HU: MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSL
MO: MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL

HU: GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG
MO: GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG

HU: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS
MO: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG
MO: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG

HU: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE
MO: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE

HU: GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS
MO: GQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS

HU: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR
MO: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU: SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR
MO: SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR

HU: WPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
MO: WHSCQKKFARSDELVRHHNMHQRNMTKLHVAL

Fig. 1

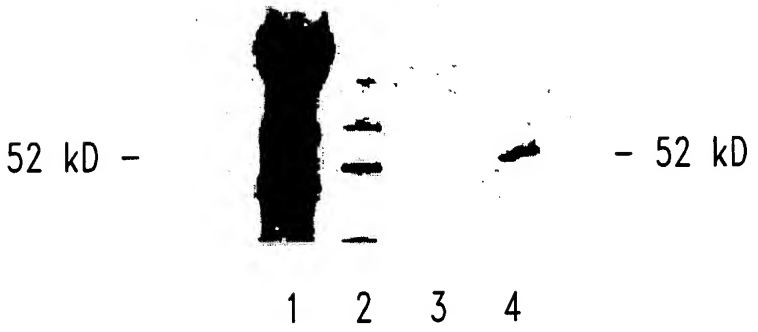


Fig. 2

52 kD
52 kD
1 2 3 4 5 6
control antibody
non immunized B6 sera
immunized B6 sera

Fig. 3

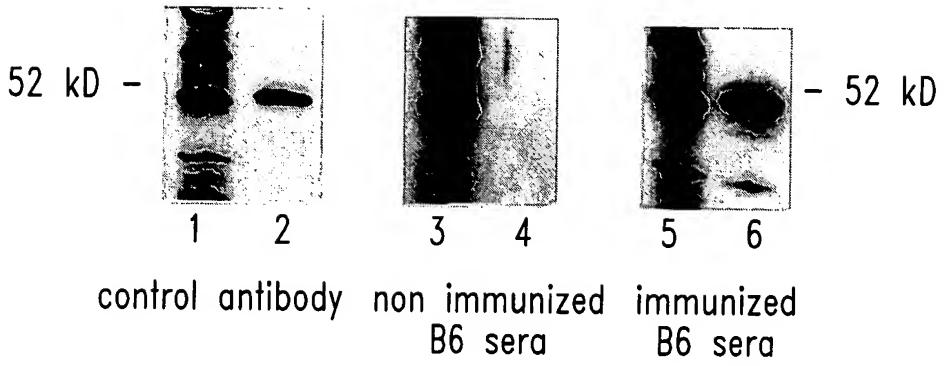

52 kD
52 kD
1 2
3 4
5 6
control antibody
non immunized B6 sera
immunized B6 sera

Fig. 4

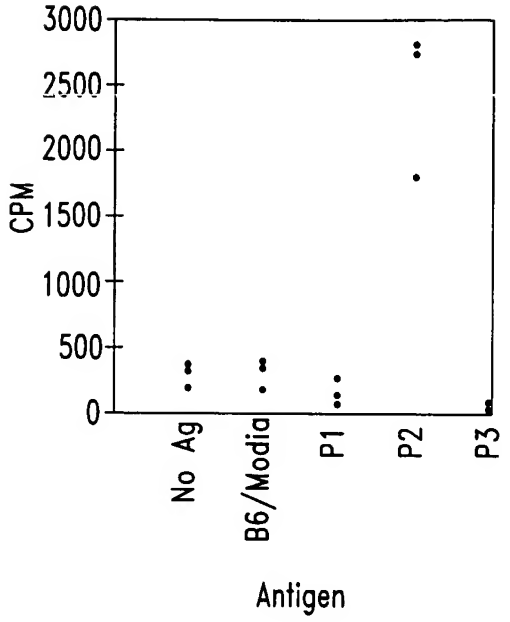


Fig. 5A

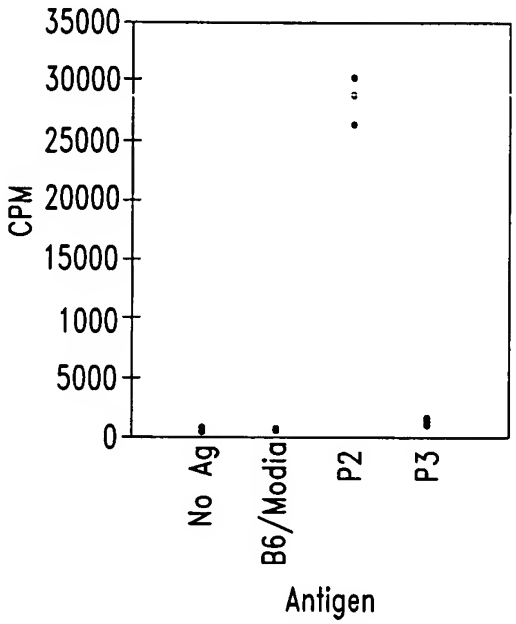


Fig. 5B

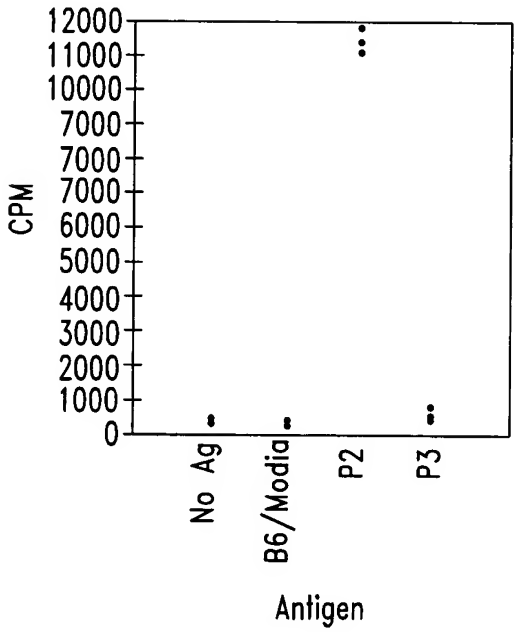


Fig. 5C

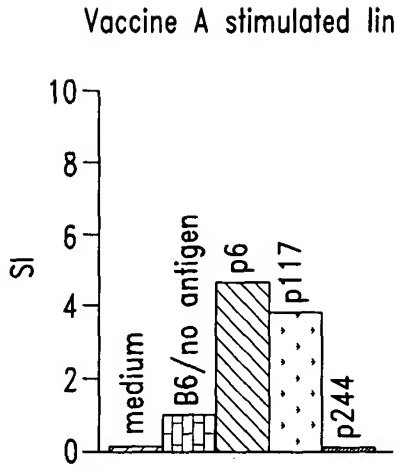

Vaccine A stimulated line
SI
10
8
6
4
2
0
medium
B6/no antigen
p6
p117
p244

Fig. 6A

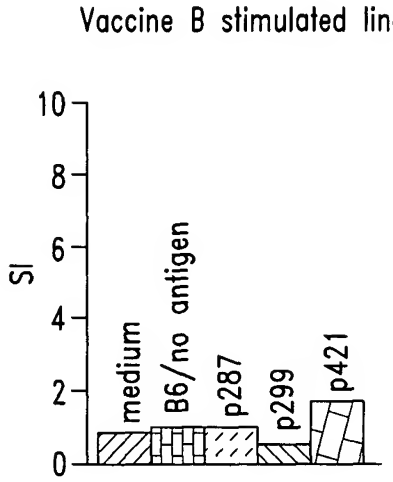

Vaccine B stimulated line
SI
10
8
6
4
2
0
medium
B6/no antigen
p287
p299
p421

Fig. 6B

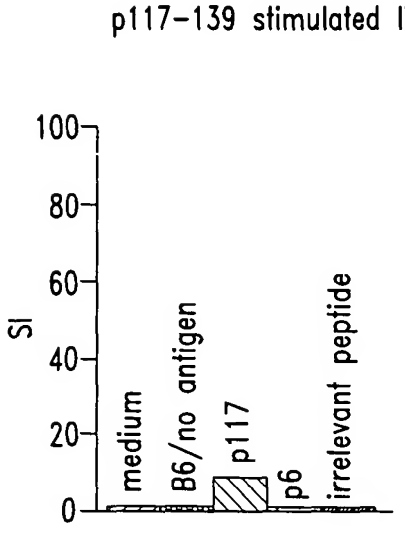


Fig. 7A

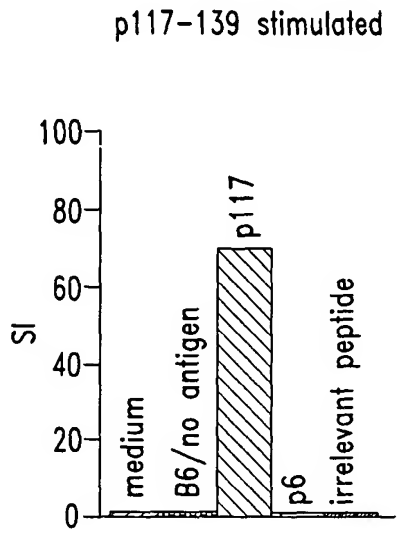


Fig. 7B

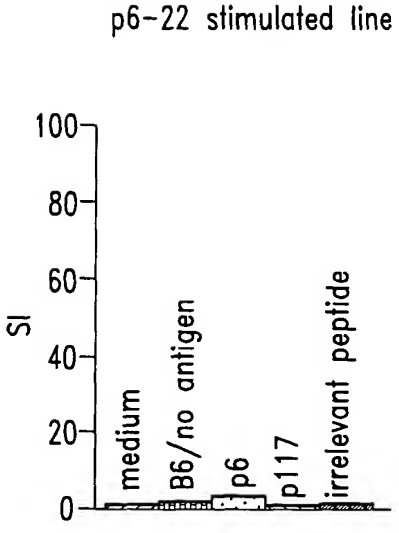


Fig. 7C

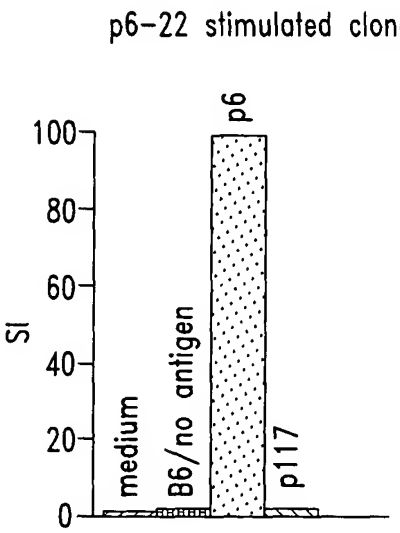


Fig. 7D

```
    5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAA.......AAAAAA.........AAAAAAAAAAA.....................
.............................RRRR.....................................
.....................................................................
.....................................................................

   80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYS
..............AAA......AAAA.....................AAA......AAAAAA..........
..........................RRRR.....................RRRRR.................
.....................................DDDDDDDDD.....................
.....................................................................

  155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGTPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
..............AAAAA.............................AAAAAA..............AA
..............RRRR.....................................................
.....................................................DDDDDDDDDDDDDD...
.....................................................................

  230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTEGQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA............AAA.AAA.....................................AAAAAAAAAAA
.................RRRRRRRRRR.....RRRR.........................RRRR.....
DDDDDD..............DDDDDDDDDDD.....................................
.....................................................ddddd.........

  305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVPGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAAA.....................................AAAA.AAAAAAAAA.
....RRRRR.....................RRRR.....................................
.......DDDDDD.....................................................
.....................................................................

  380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
.............AAAA.AAAA..AA.....AAAA..........AAA....AAAAAAAA...AAA........
.................................................RRRR..RRRR...............
.....................................................................
..................ddddddddddddd.....................................
```

Fig. 8A

```
    5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAA.......AAAAAA.........AAAAAAAAAAAA.....................
.............................RRRR.....................................
.....................................................................
.....................................................................

   80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTLHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYS
.........................AAAA.....................AAA......AAAAAA...........
.........................RRRR.....................RRRRR.....................
.........................................DDDDDDDDD..........................
.....................................................................

  155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGAPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
.................AAAAA.............................AAAAAA.................AA
.................RRRR.....................................................
.....................................................DDDDDDDDDDDDDD...
.....................................................................

  230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTEGQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA.............AAA.AAA.....................................AAAAAAAAAAA
.....................RRRRRRRRRR.....RRRR.............................RRRR.....
DDDDDD................DDDDDDDDDDD.....................................
.....................................................ddddd.........

  305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVSGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAAA.....................................AAAA.AAAAAAAAA.
....RRRRR.......................RRRR.....................................
..DDDDDDDDDD.....................................................
.....................................................................

  380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWHSCQKKFARSDELVRHHNMHQRNMTKLHVAL
.............AAAA.AAAA..AA.....AAAA...........AA....AAAAAAAA...AAAA.......
.............................................RRRR..RRRR.................
.....................................................................
.................ddddddddddd.....................................
```

Fig. 8B

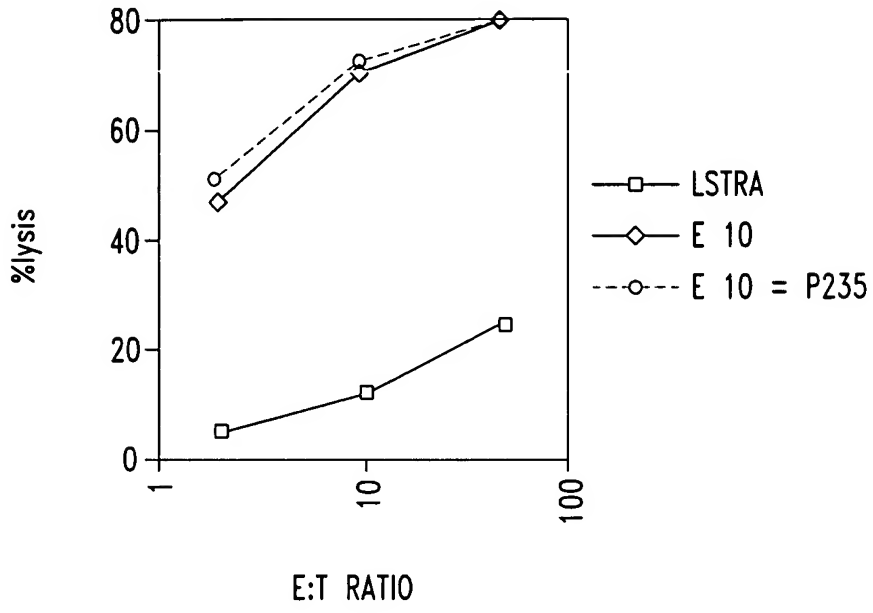

80
60
40
20
0
%lysis
1
10
100
E:T RATIO
LSTRA
E 10
E 10 = P235

Fig. 9A

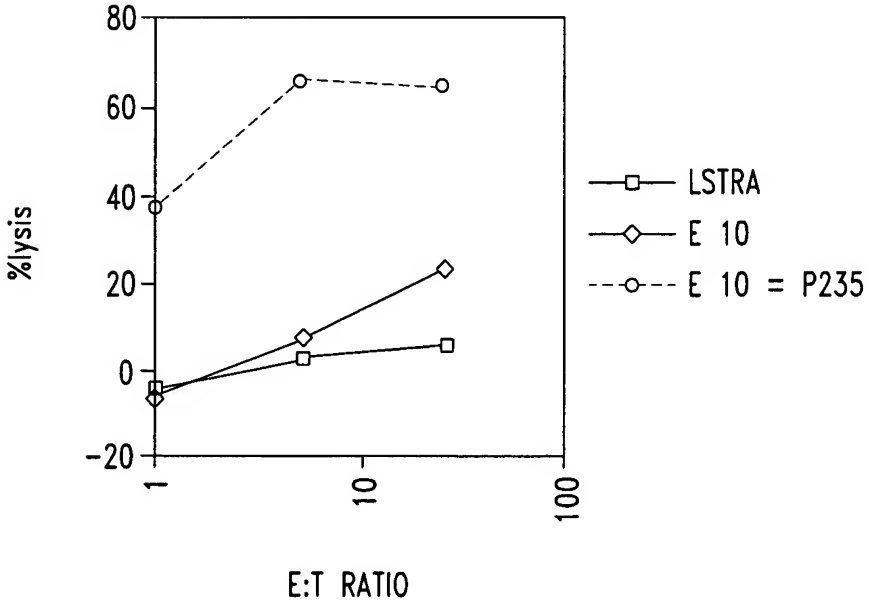

80
60
40
20
0
-20
%lysis
1
10
100
E:T RATIO
LSTRA
E 10
E 10 = P235

Fig. 9B

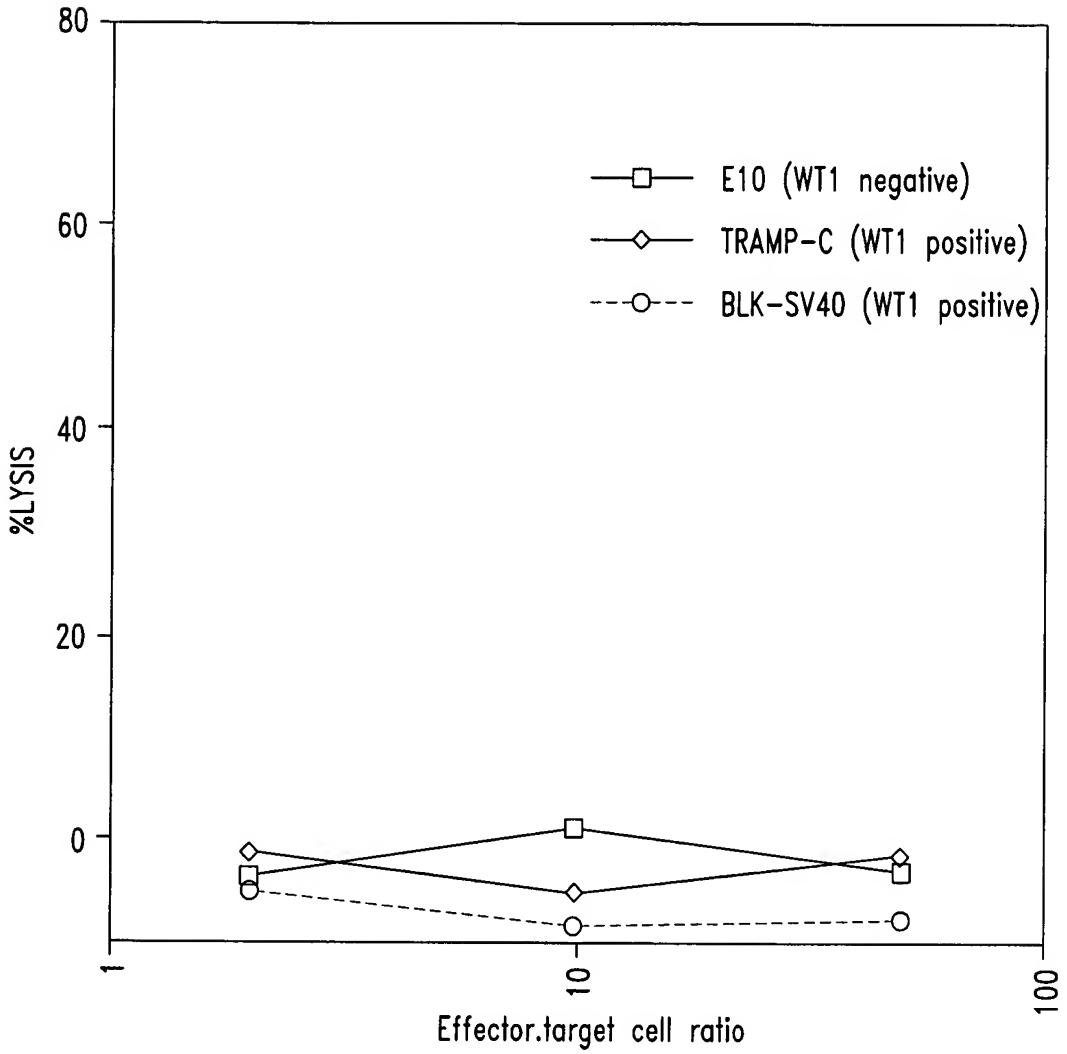

80
60
40
20
0
%LYSIS
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
1
10
100
Effector.target cell ratio

Fig. 10A

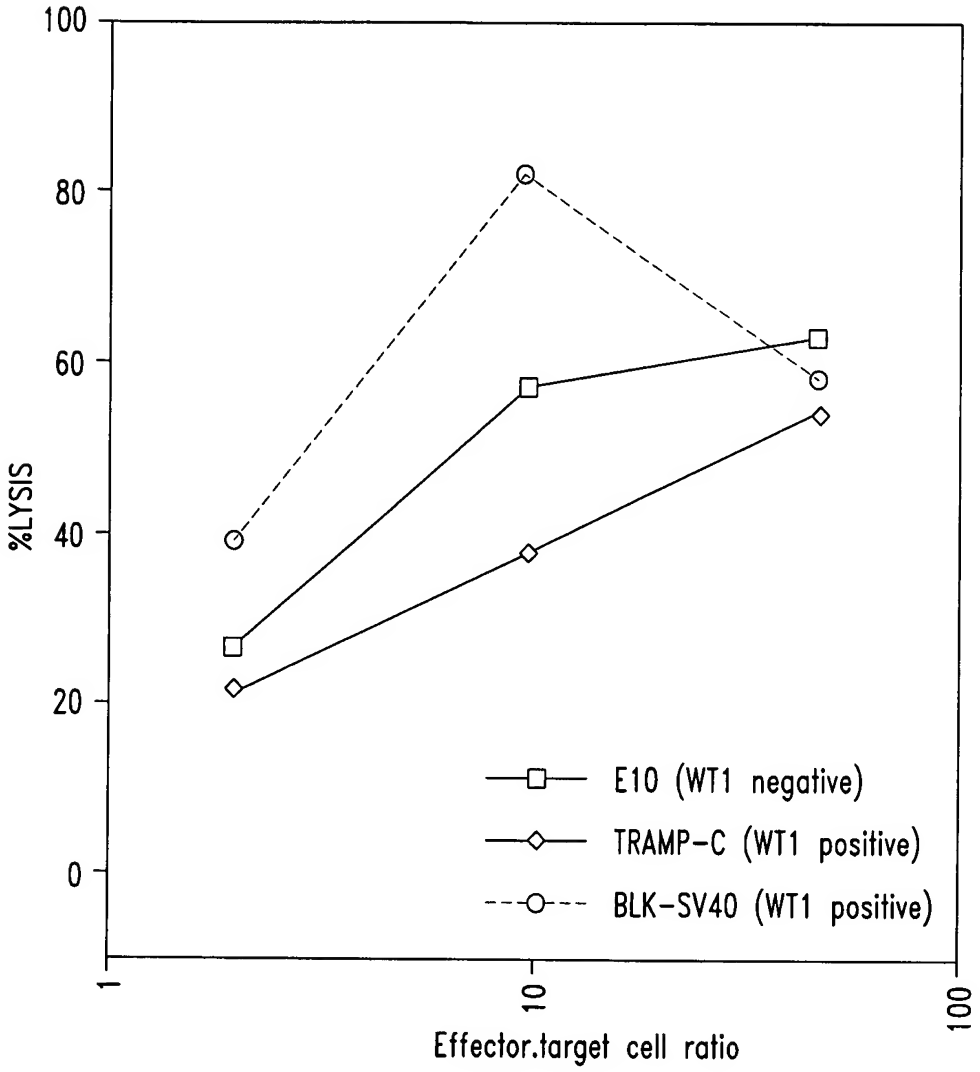

100
80
60
40
20
0
%LYSIS
1
10
100
Effector.target cell ratio
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)

Fig. 10B

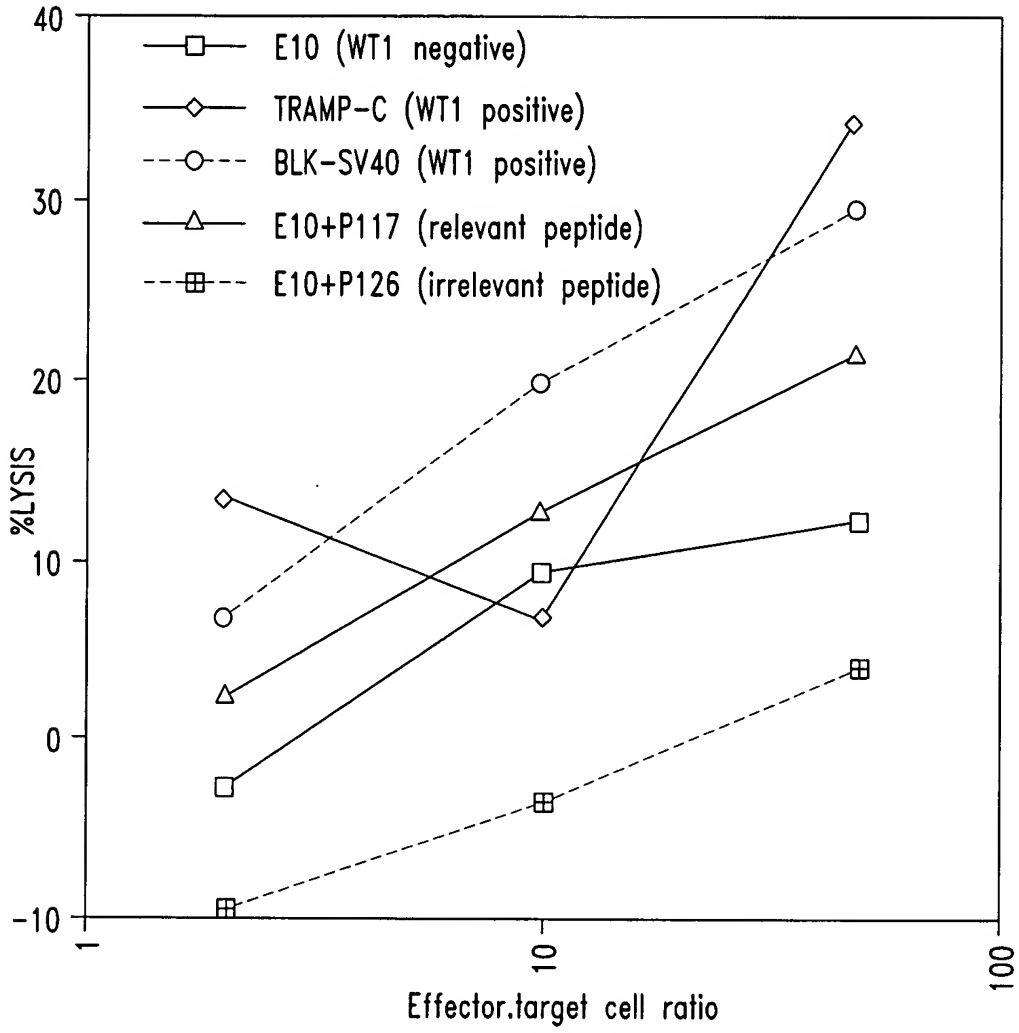

E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelevant peptide)
%LYSIS
40
30
20
10
0
-10
1
10
100
Effector.target cell ratio

Fig. 10C

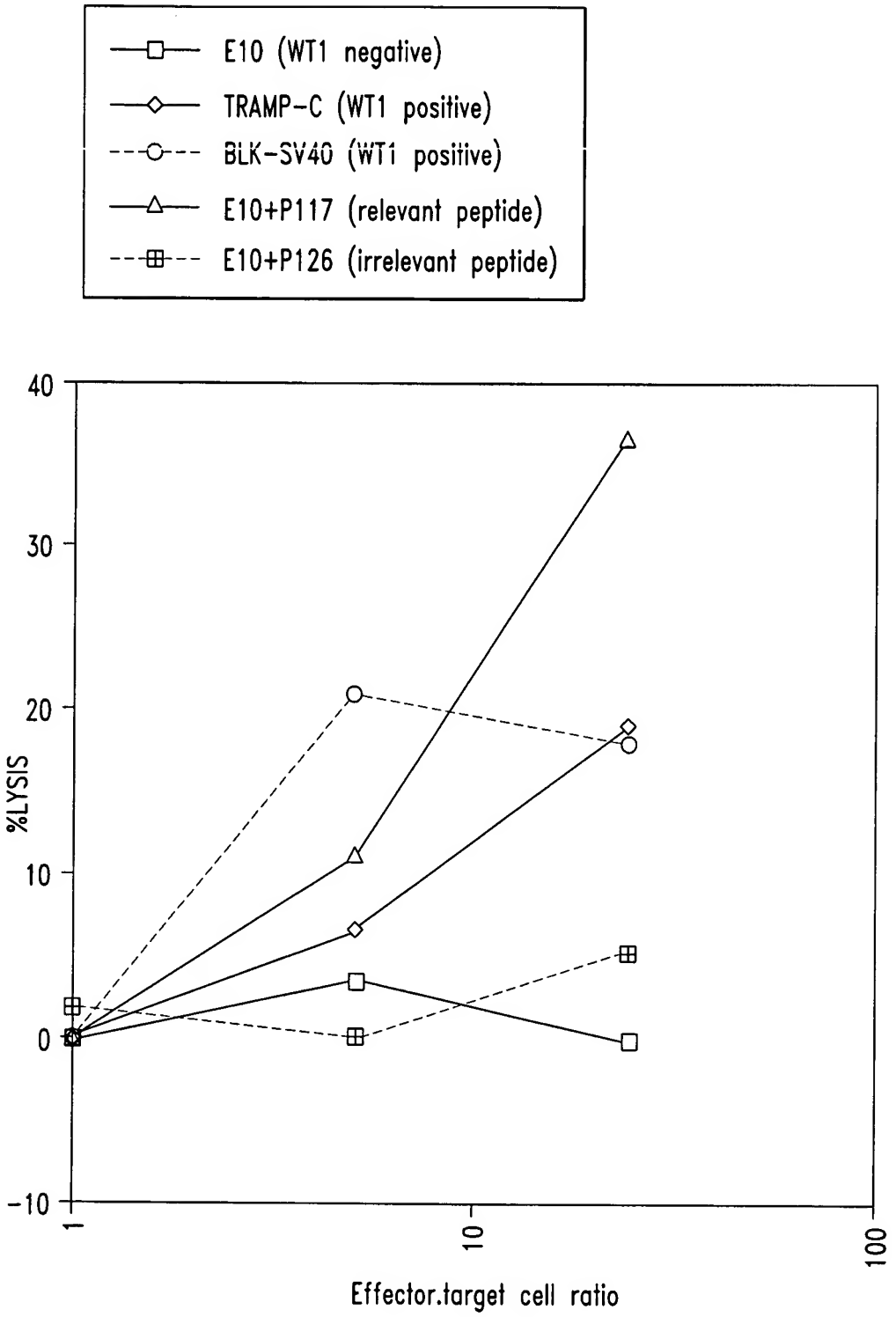

E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelevant peptide)
40
30
20
10
0
-10
%LYSIS
1
10
100
Effector.target cell ratio

Fig. 10D

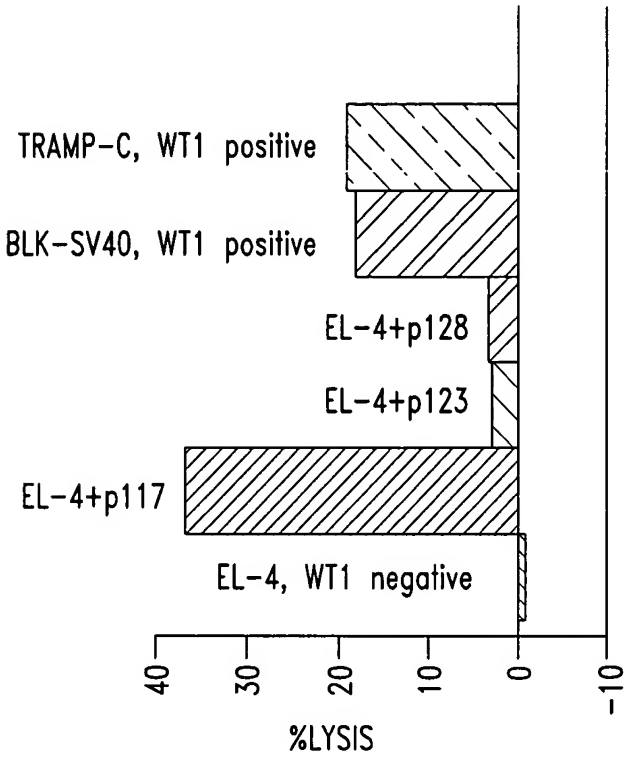


Fig. 11B

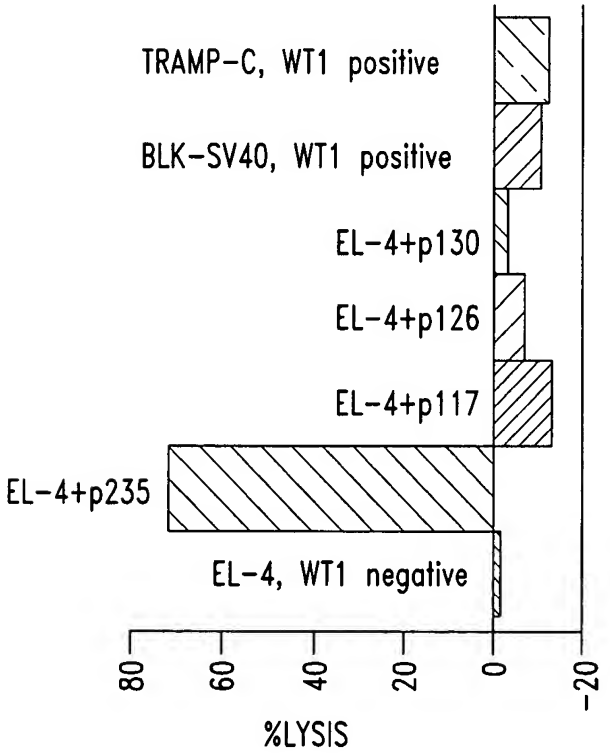


Fig. 11A

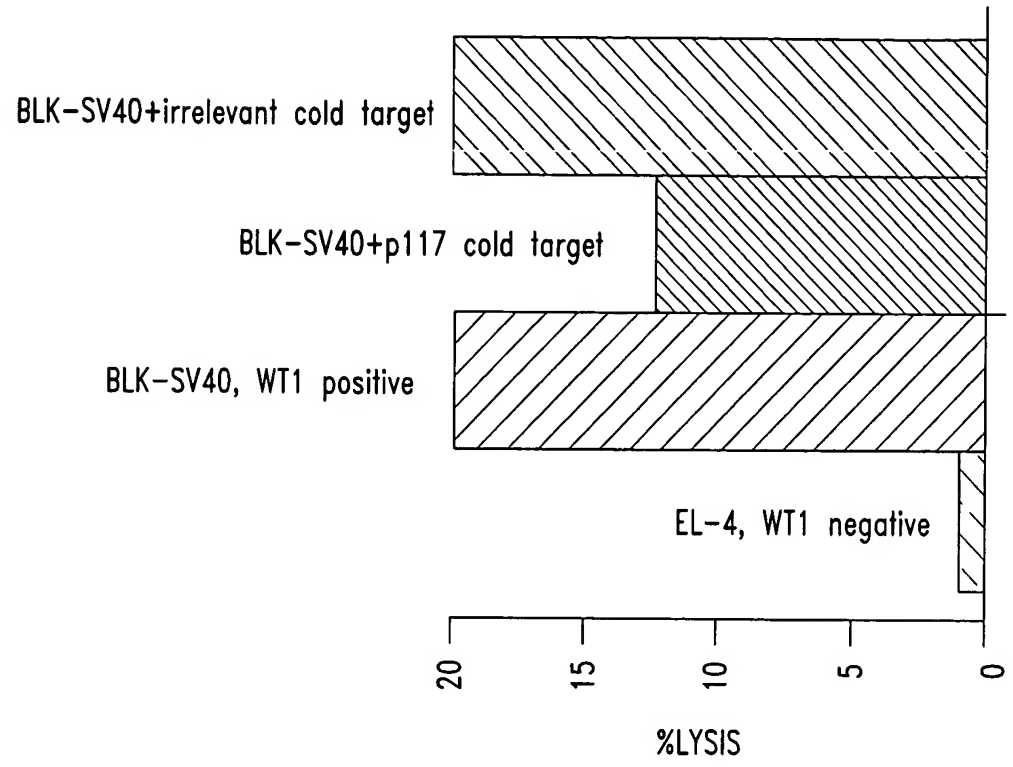


Fig. 12B

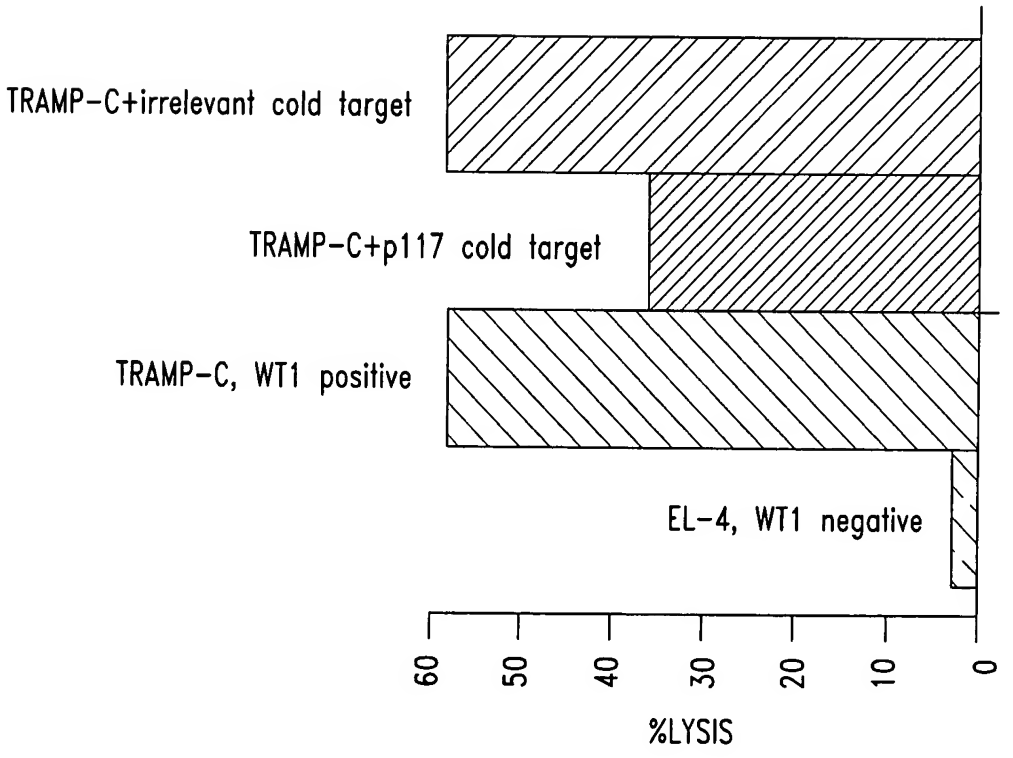


Fig. 12A

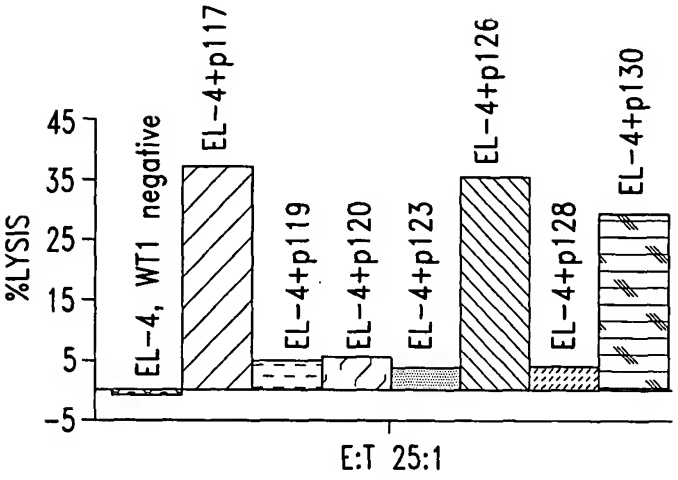


Fig. 13A

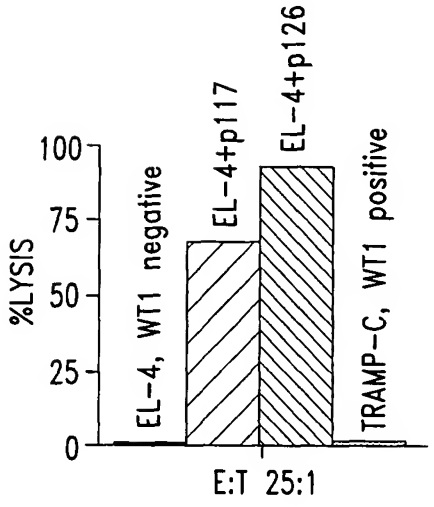


Fig. 13B

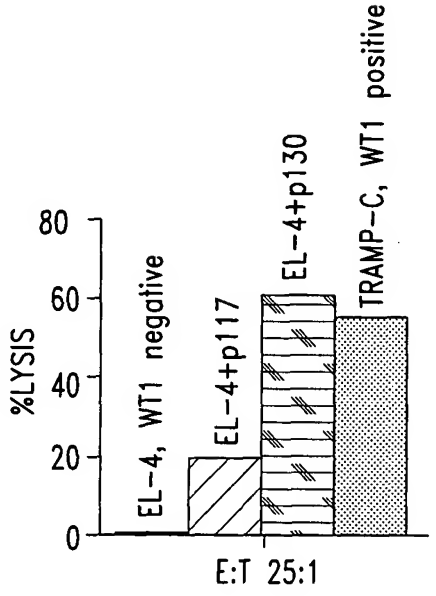


Fig. 13C

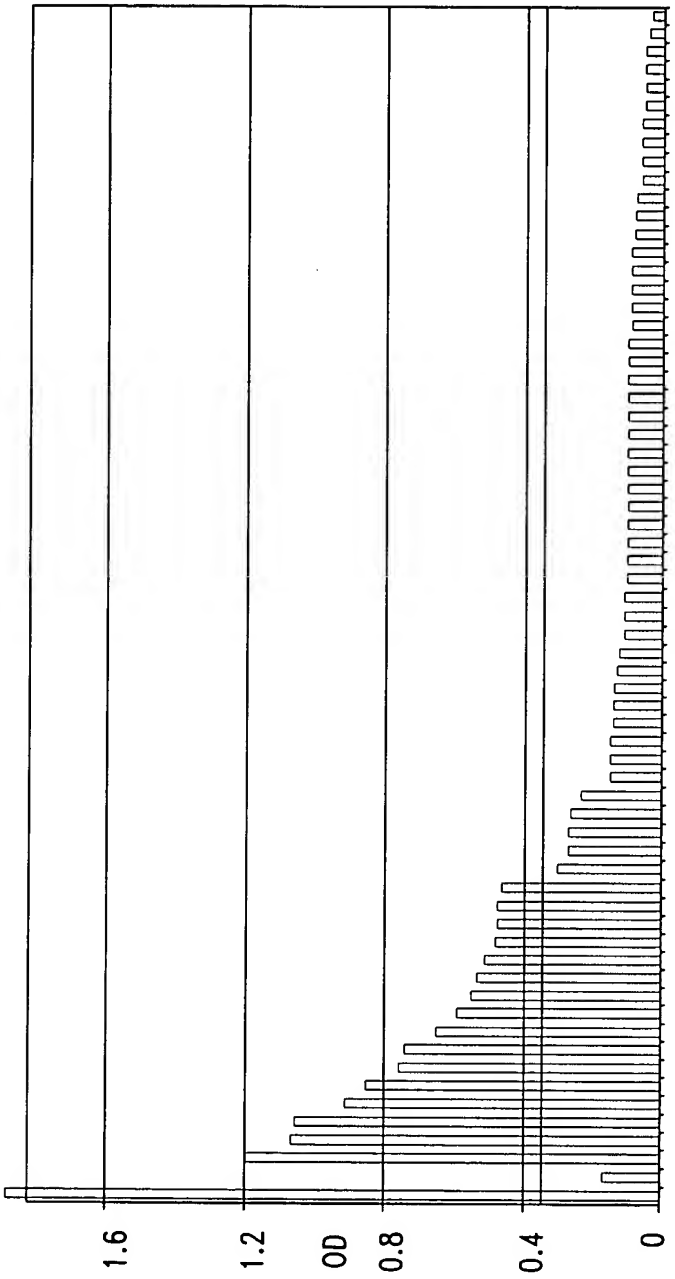


Fig. 14

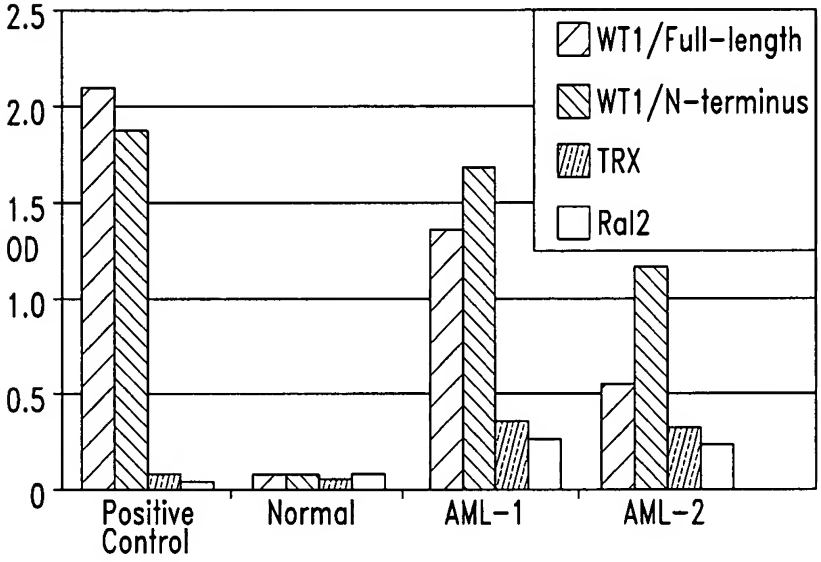

2.5
2.0
1.5
OD
1.0
0.5
0
WT1/Full-length
WT1/N-terminus
TRX
Ral2
Positive Control
Normal
AML-1
AML-2

Fig. 15

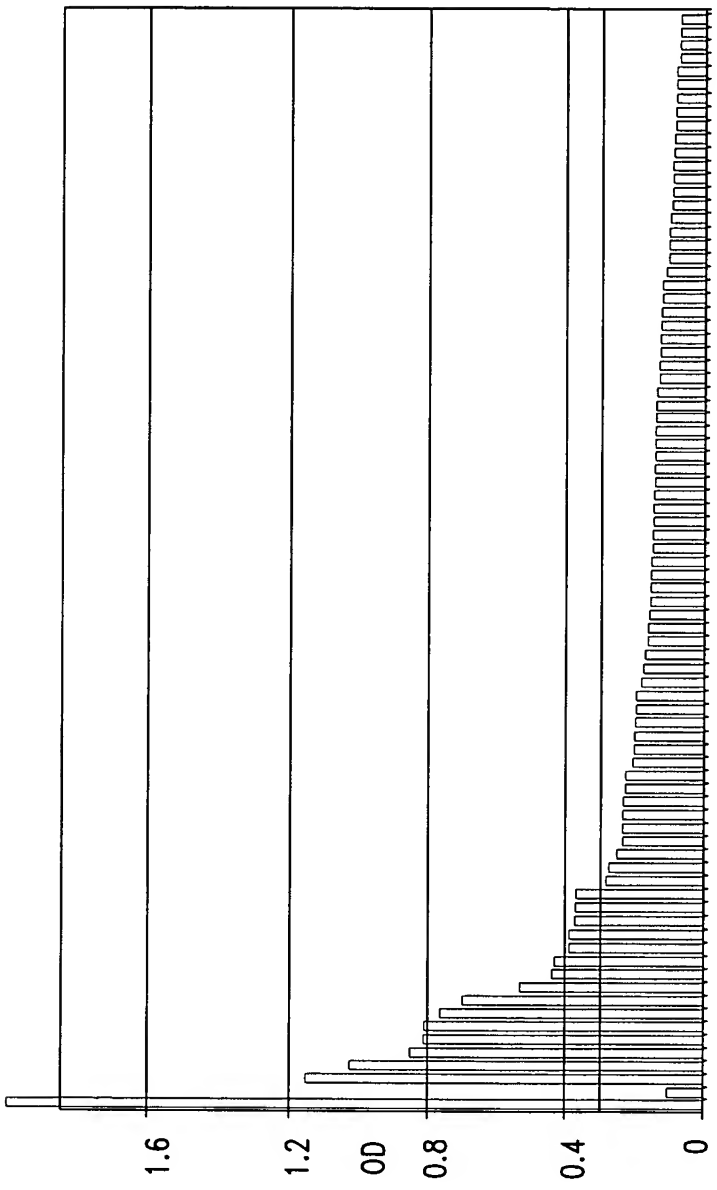


Fig. 16

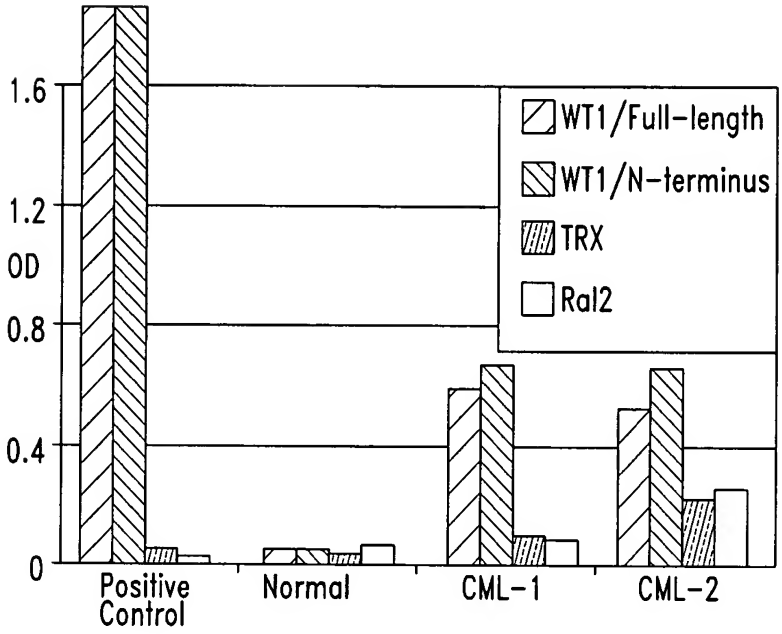

1.6
1.2
OD
0.8
0.4
0
WT1/Full-length
WT1/N-terminus
TRX
Ral2
Positive Control
Normal
CML-1
CML-2

Fig. 17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

NAME	Recombinant Protein	WT1 Amino Acid Position	Molecular Weight
WT1/full-length	Ral2-WT1 full length fusion protein	aa 1-449	85kDa
WT1/N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1/C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

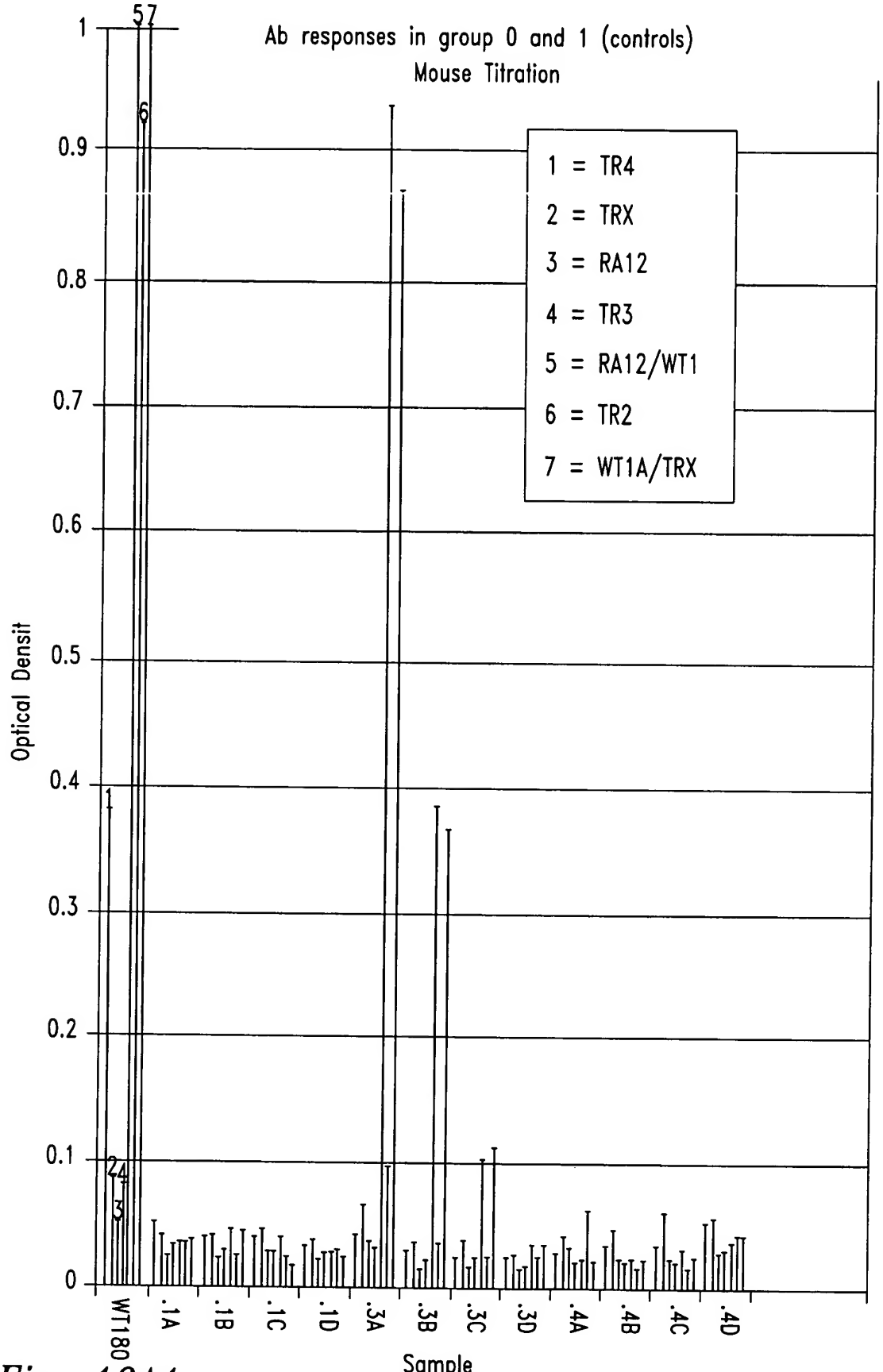


Fig. 19A1 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

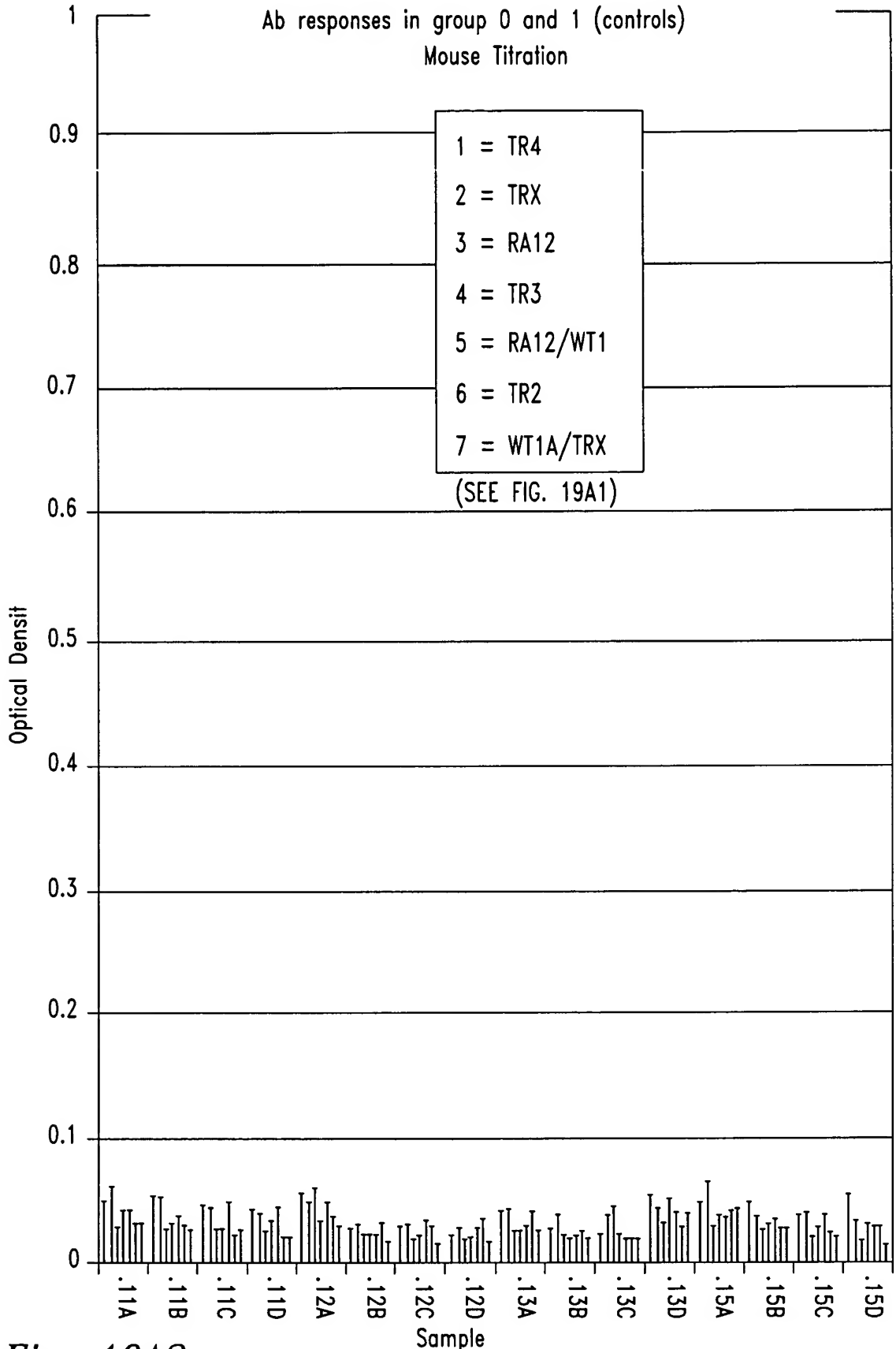


Fig. 19A2 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

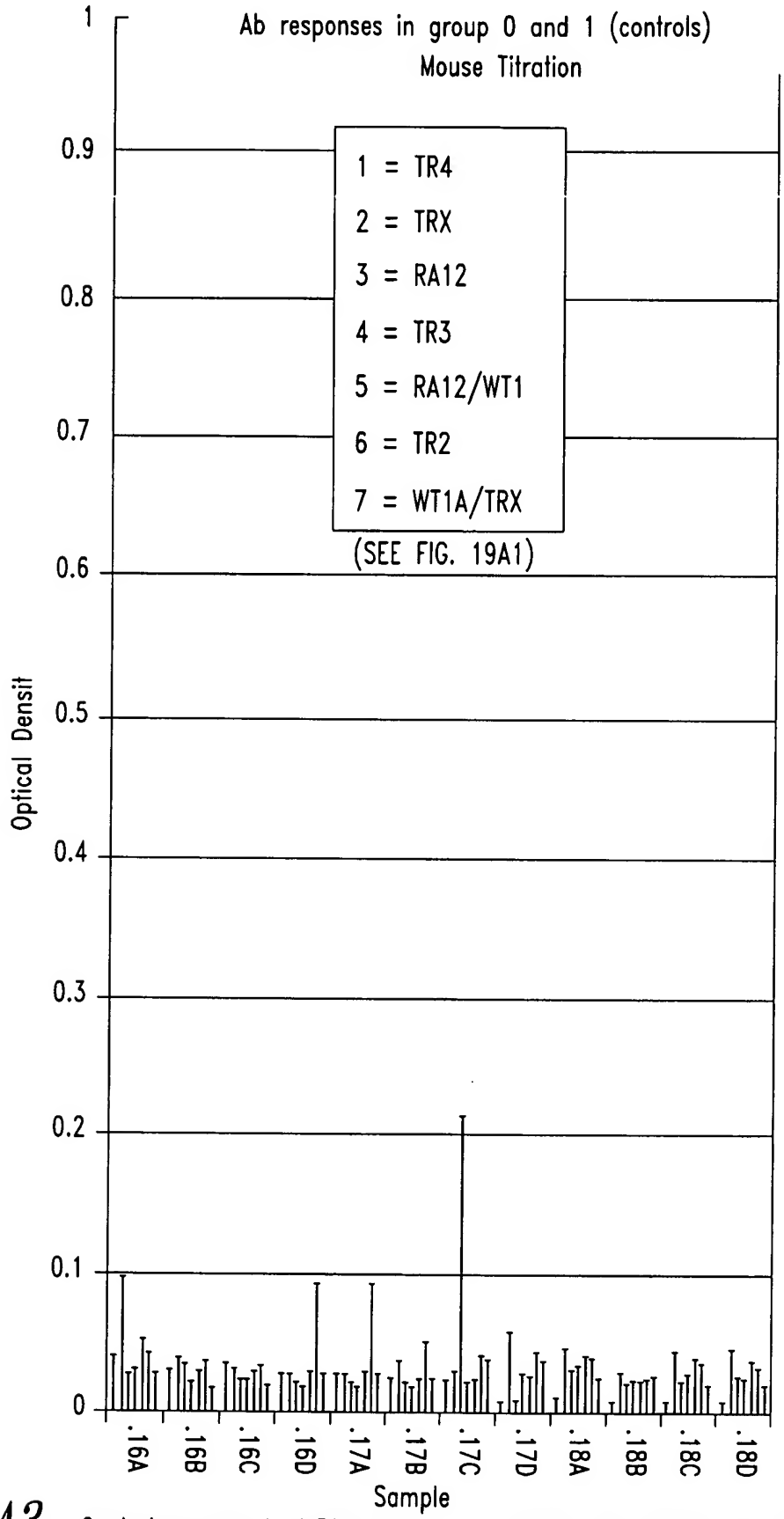


Fig. 19A3 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

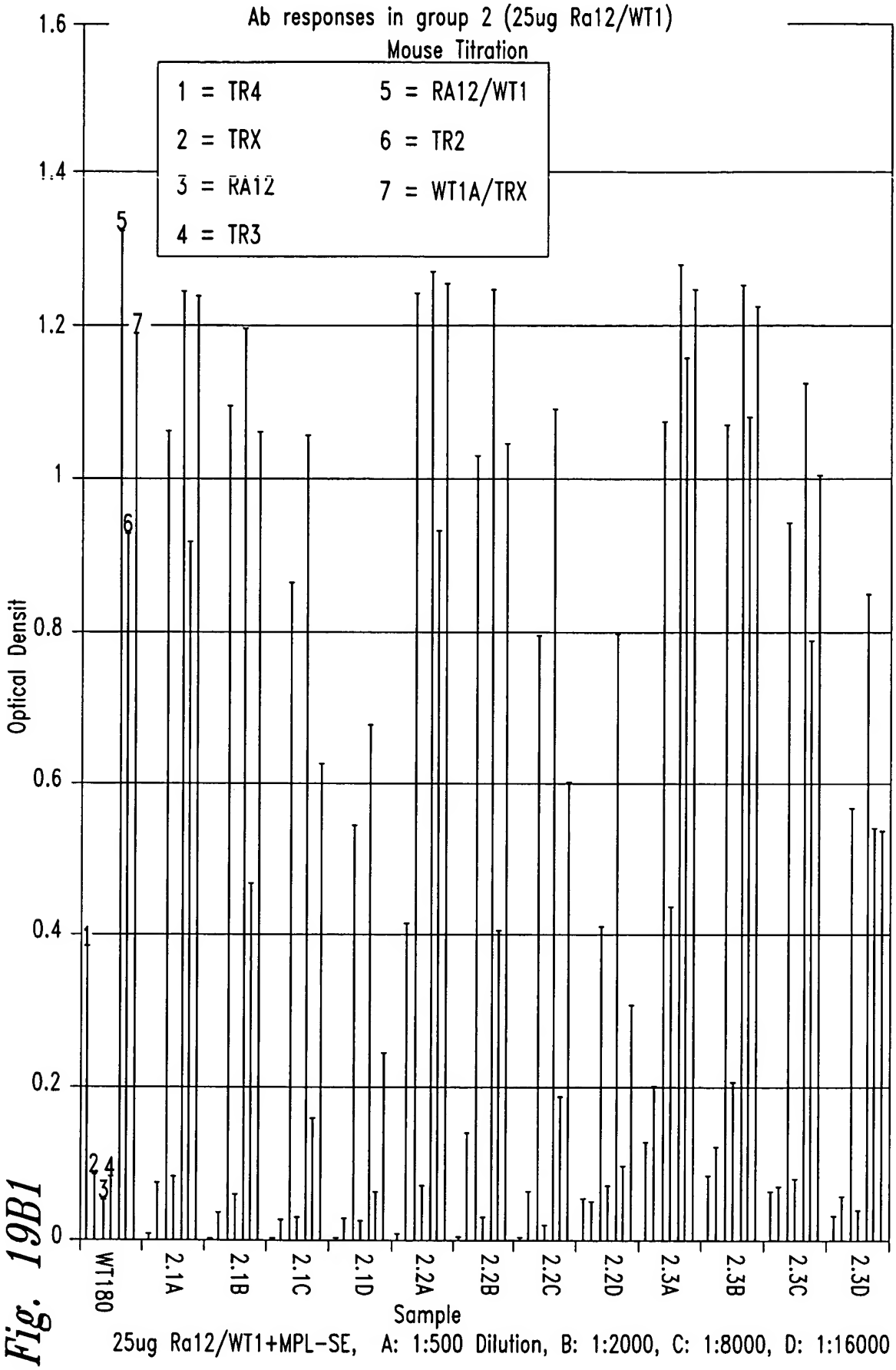

Ab responses in group 2 (25ug Ra12/WT1)
Mouse Titration
1 = TR4
2 = TRX
3 = RA12
4 = TR3
5 = RA12/WT1
6 = TR2
7 = WT1A/TRX
Optical Densit
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT180
2.1A
2.1B
2.1C
2.1D
2.2A
2.2B
2.2C
2.2D
2.3A
2.3B
2.3C
2.3D
Sample
25ug Ra12/WT1+MPL-SE, A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

Fig. 19B1

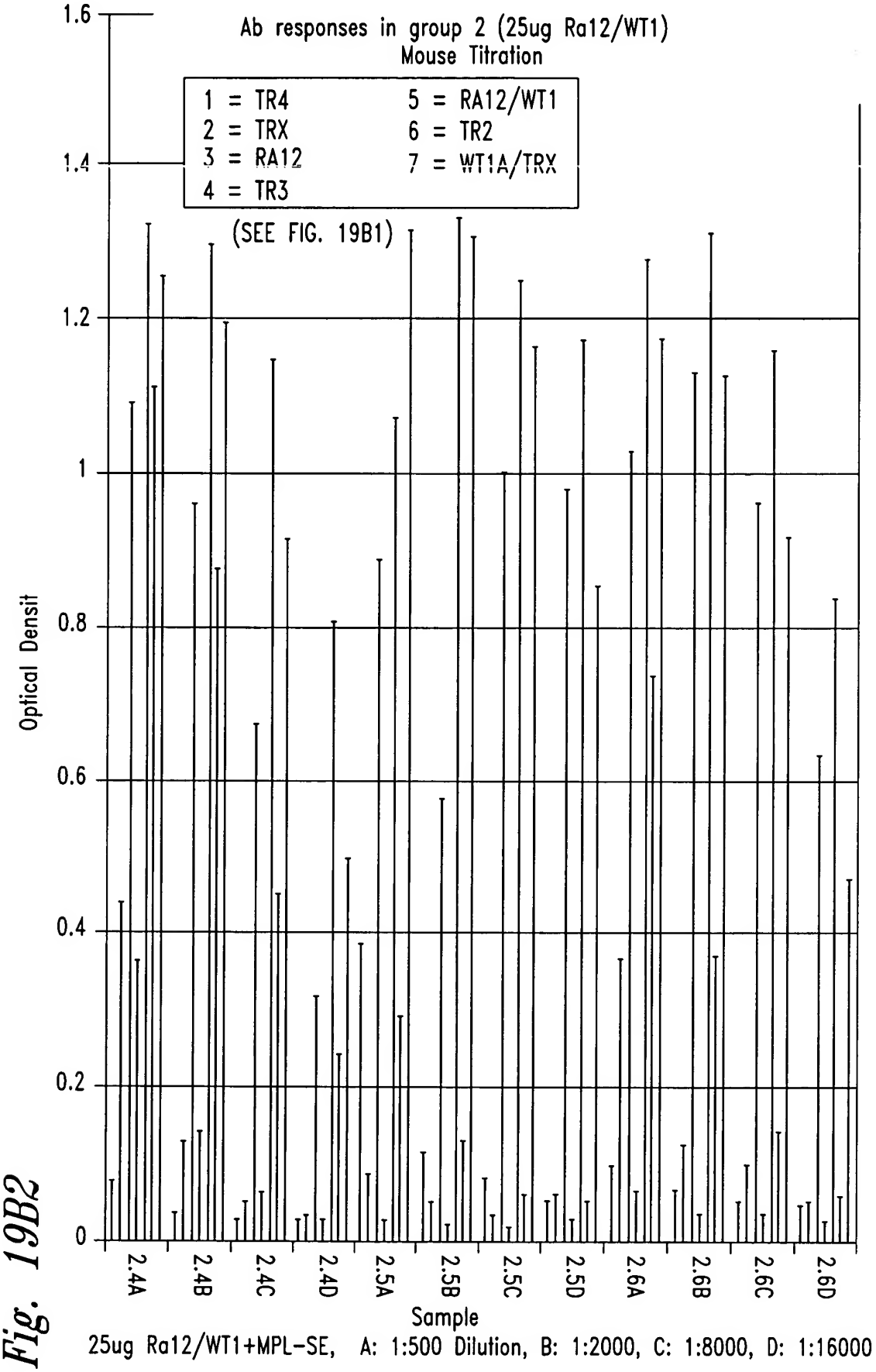

Ab responses in group 2 (25ug Ra12/WT1)
Mouse Titration
1 = TR4
2 = TRX
3 = RA12
4 = TR3
5 = RA12/WT1
6 = TR2
7 = WT1A/TRX
(SEE FIG. 19B1)
Optical Densit
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2.4A
2.4B
2.4C
2.4D
2.5A
2.5B
2.5C
2.5D
2.6A
2.6B
2.6C
2.6D
Sample
25ug Ra12/WT1+MPL-SE, A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

Fig. 19B2

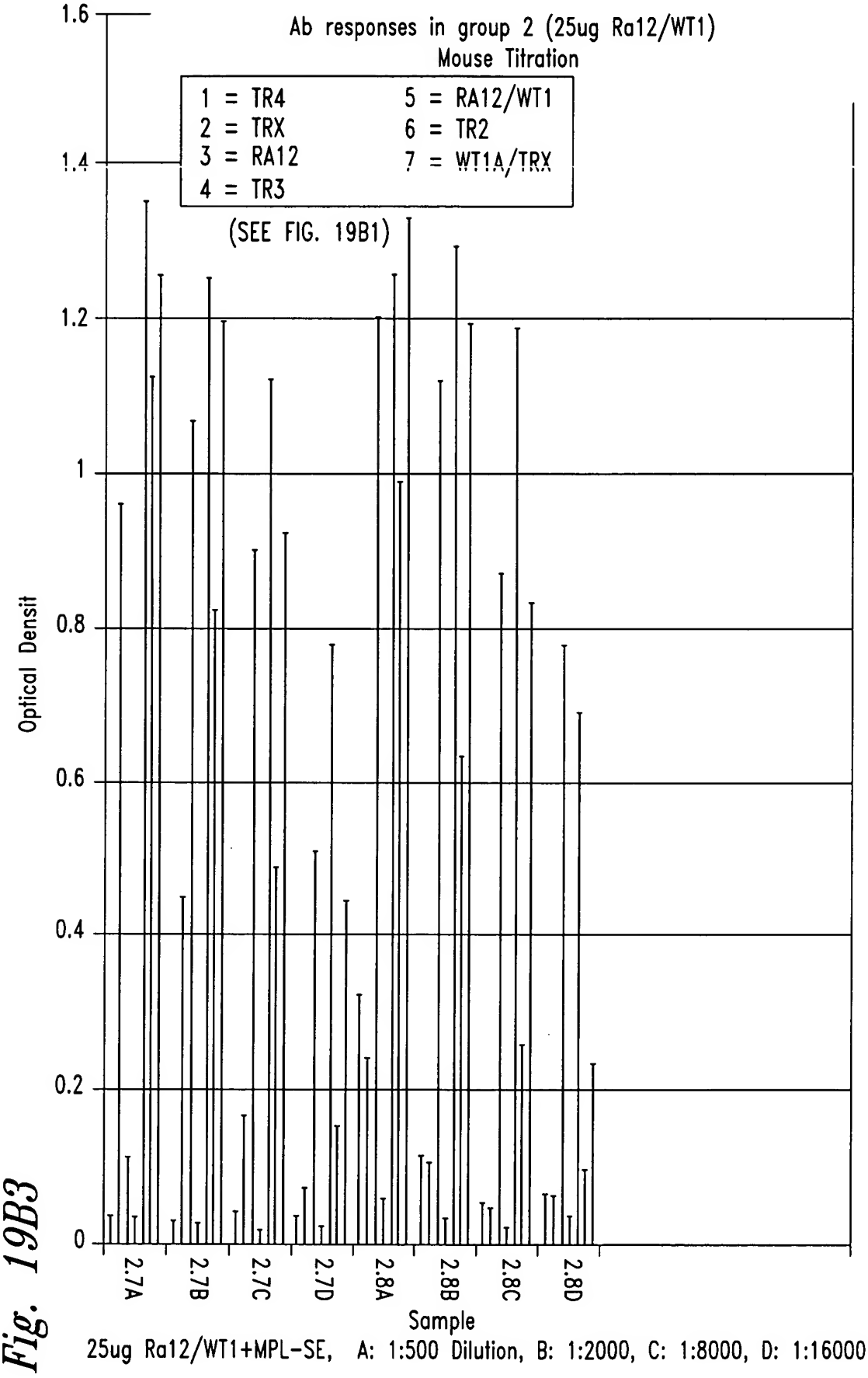

Ab responses in group 2 (25ug Ra12/WT1)
Mouse Titration
1 = TR4
2 = TRX
3 = RA12
4 = TR3
5 = RA12/WT1
6 = TR2
7 = WT1A/TRX
(SEE FIG. 19B1)
Optical Densit
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2.7A
2.7B
2.7C
2.7D
2.8A
2.8B
2.8C
2.8D
Sample
25ug Ra12/WT1+MPL-SE, A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

Fig. 19B3

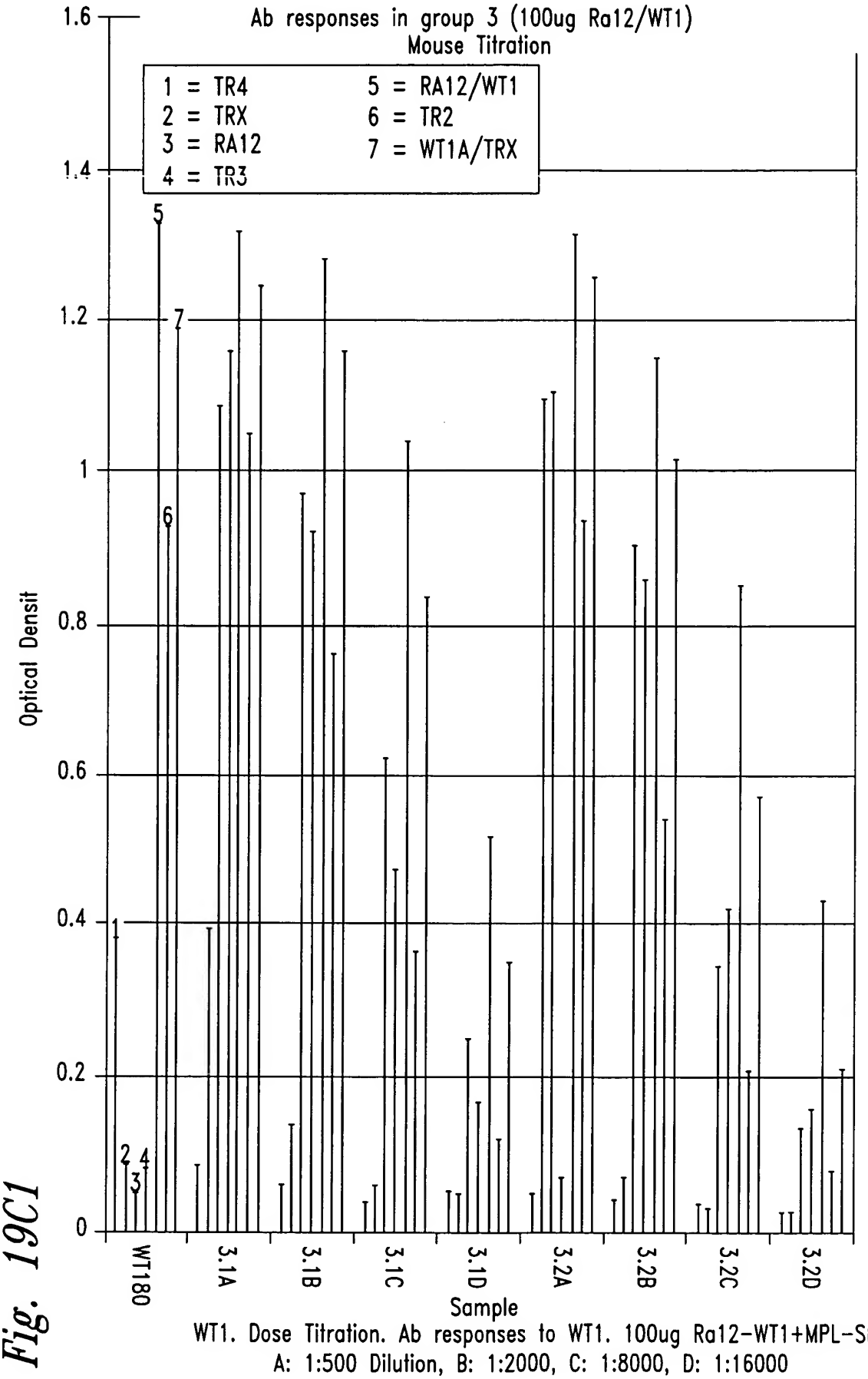


Fig. 19C1

WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

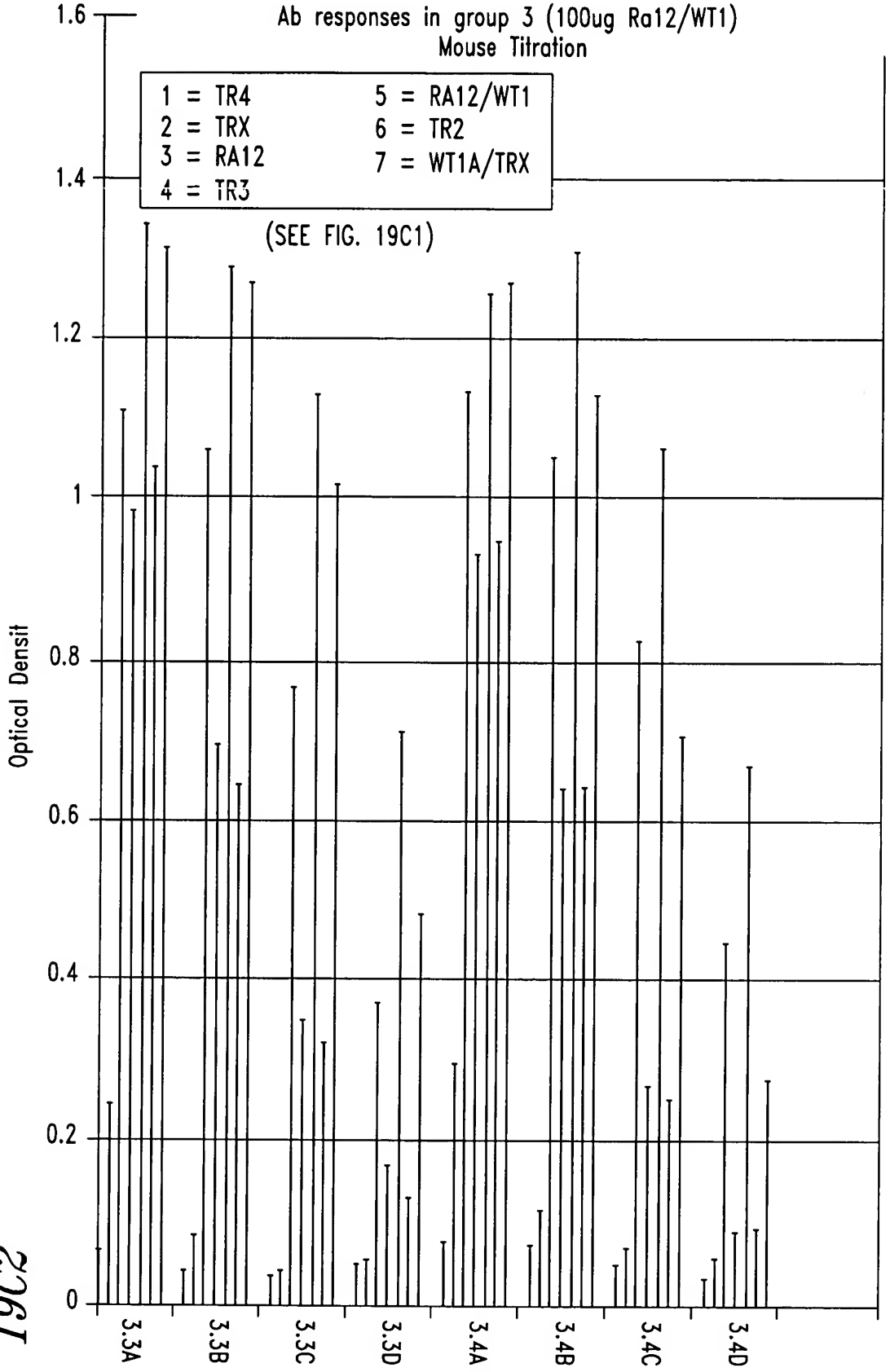


Fig. 19C2

WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

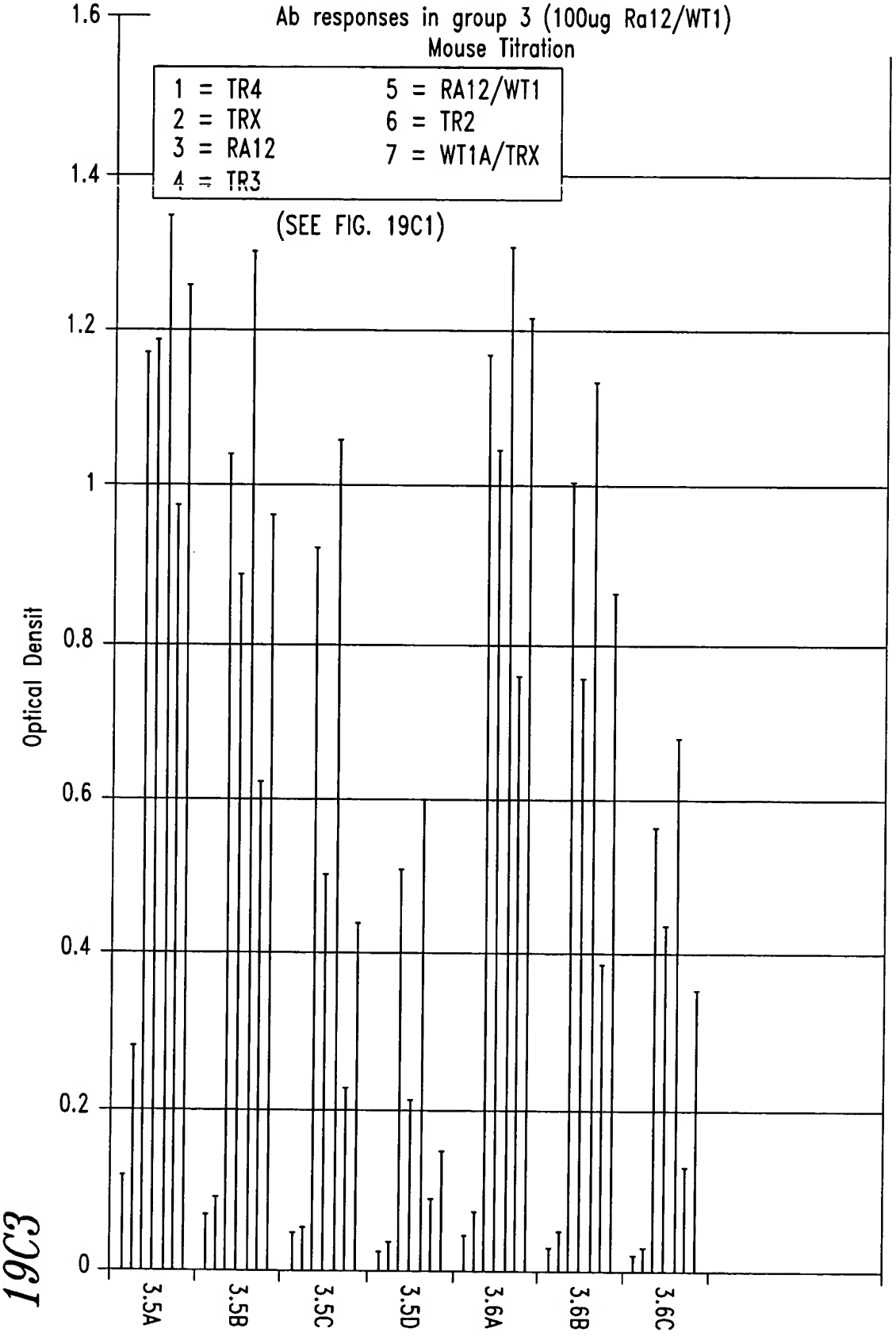


Fig. 19C3

WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

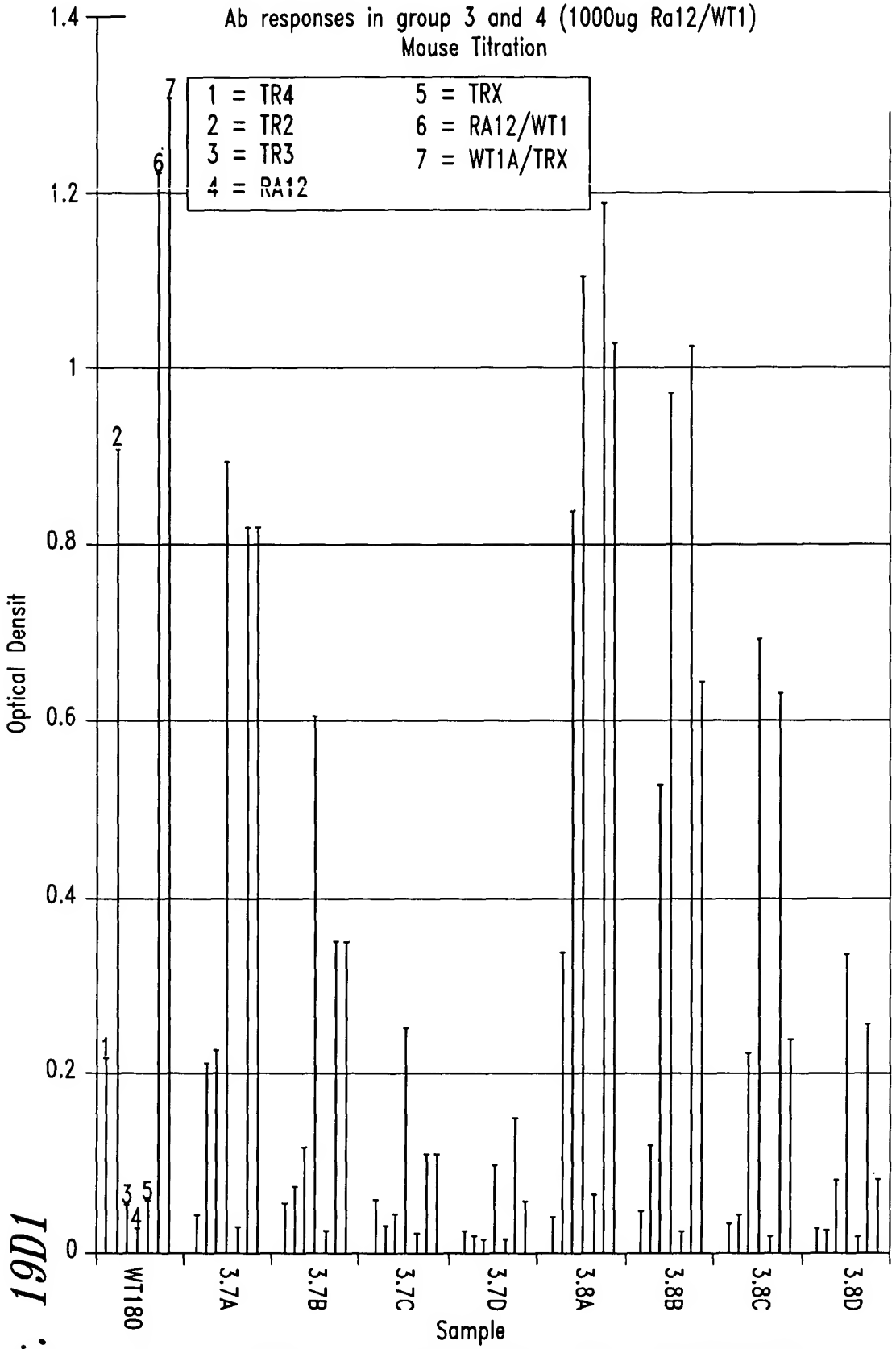


Fig. 19D1

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

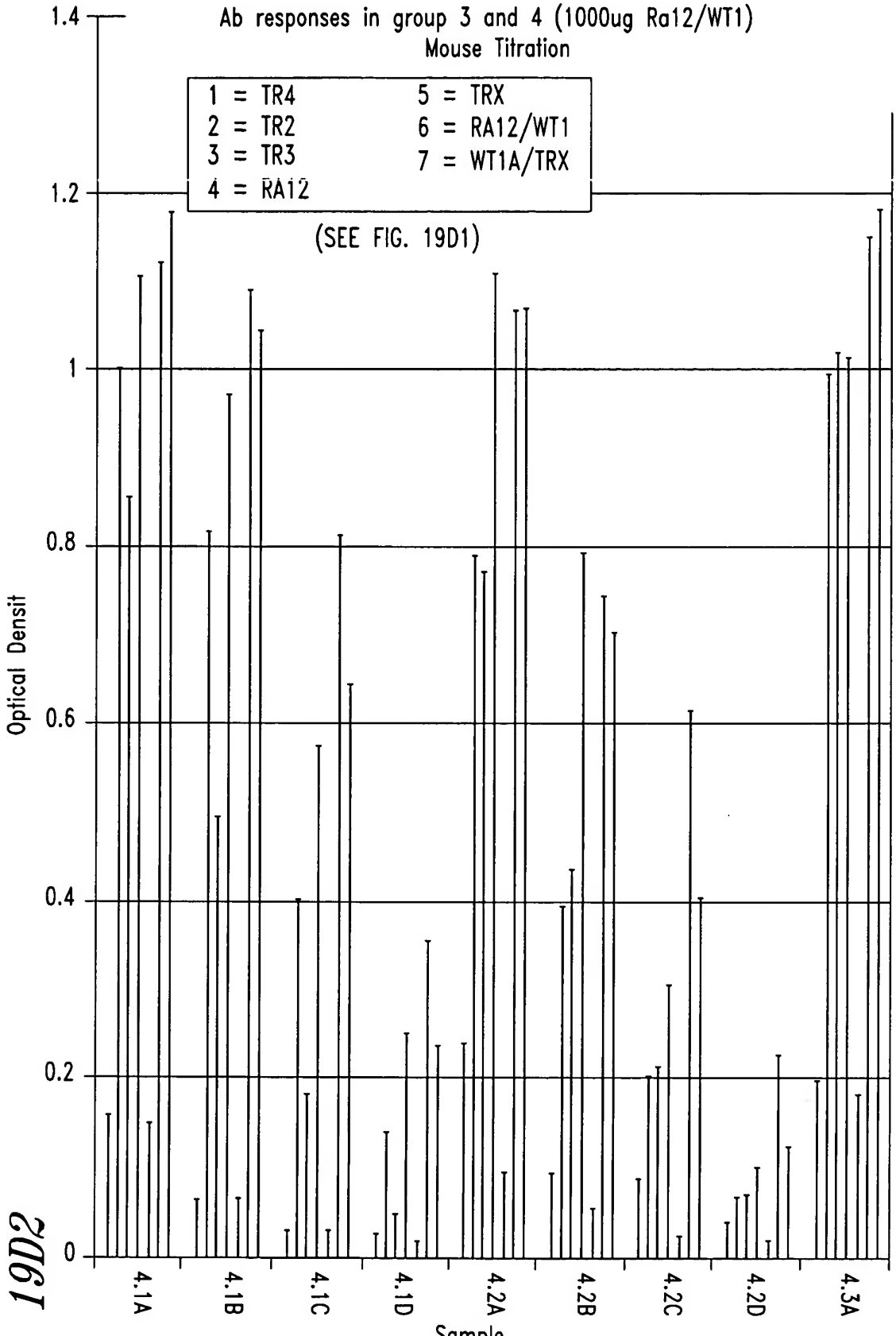


Fig. 19D2

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

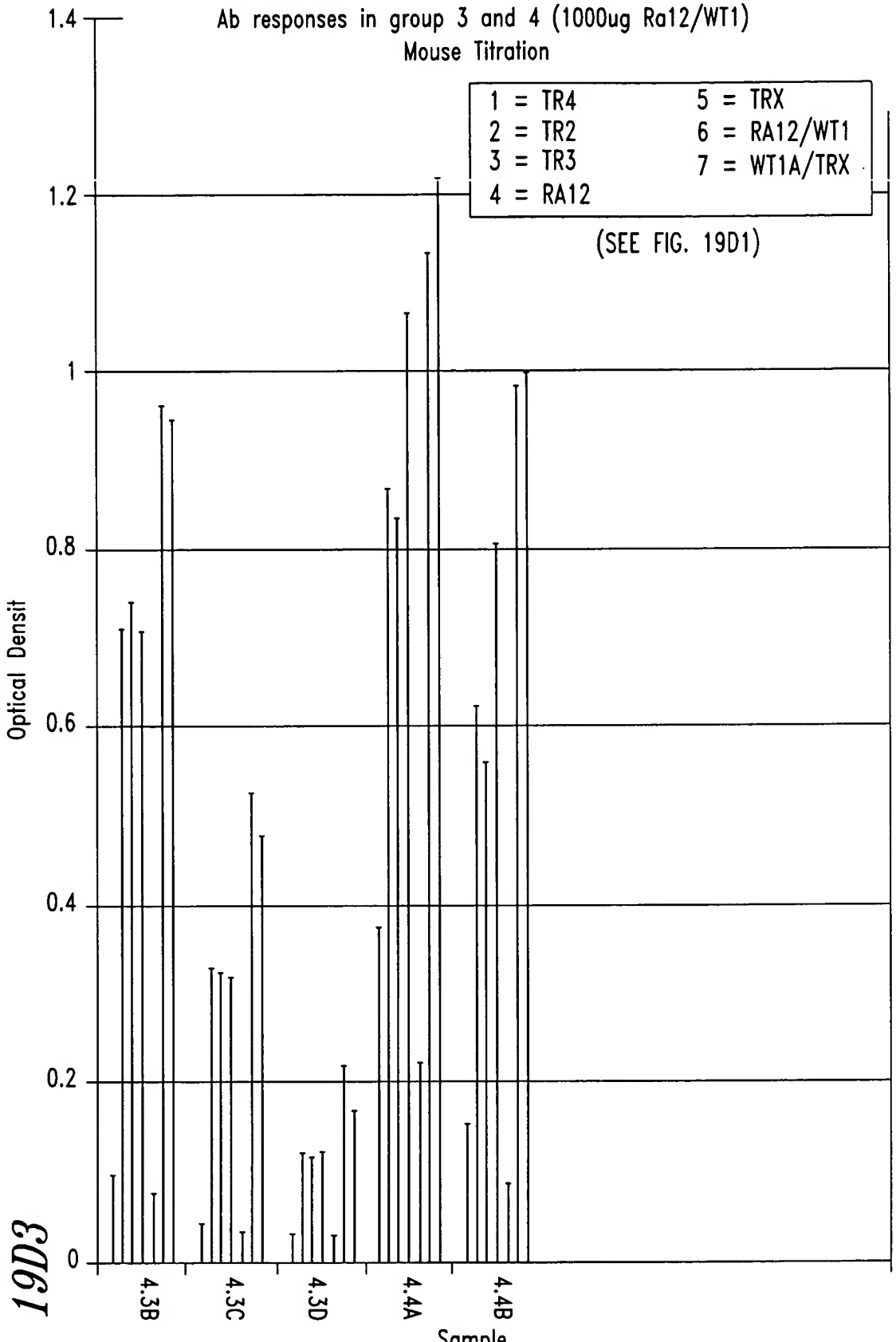
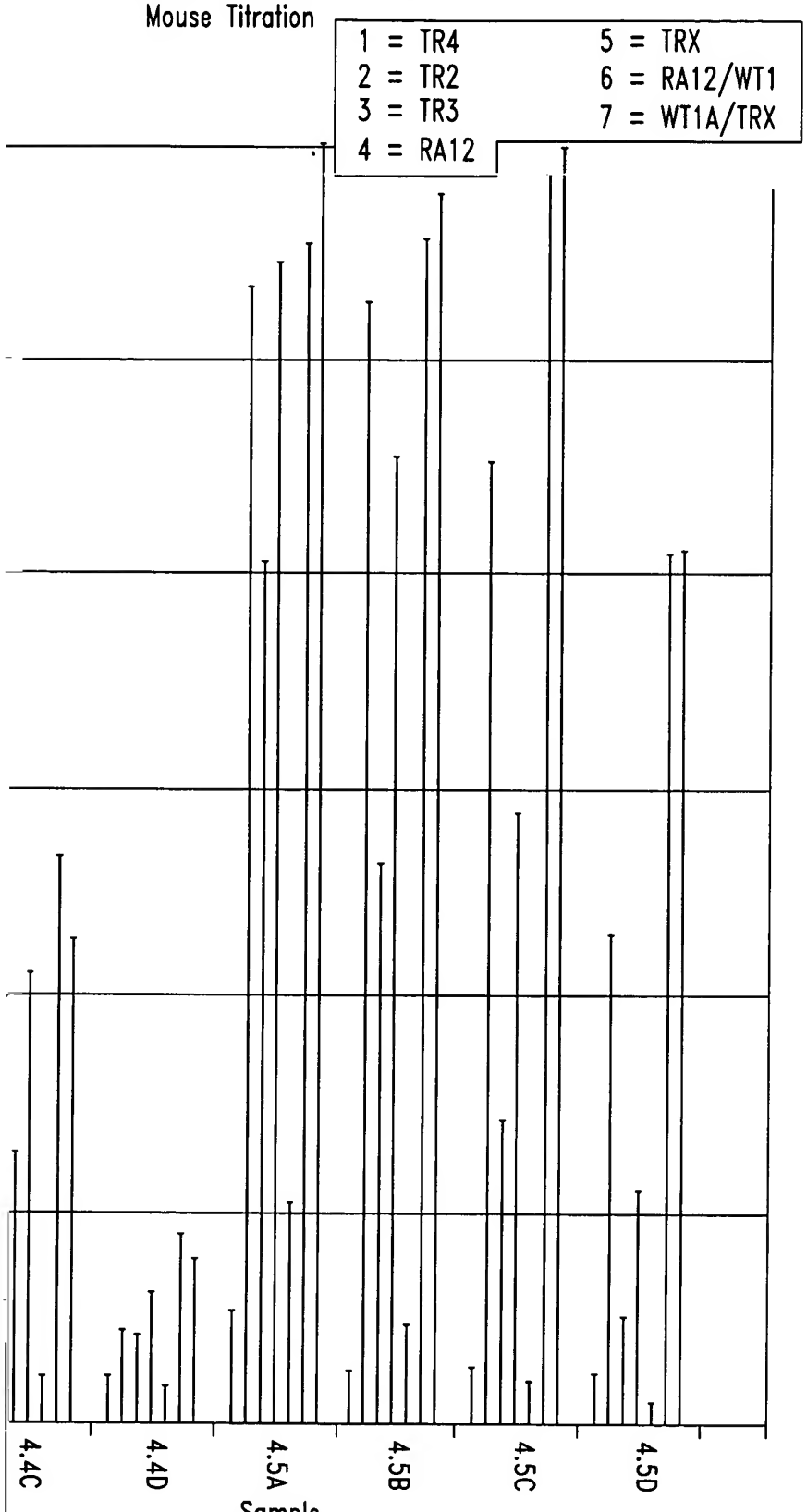


Fig. 19D3

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

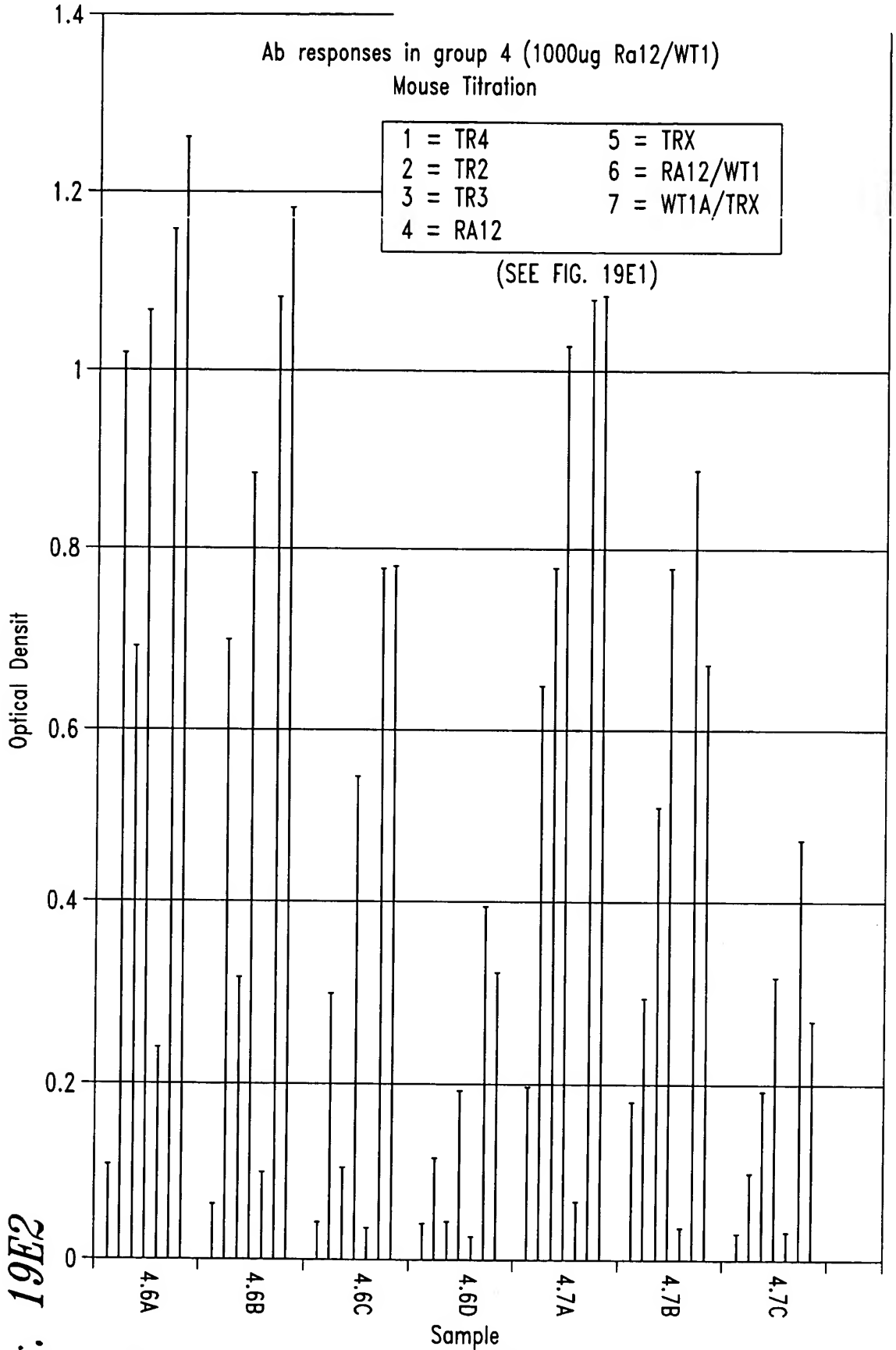


Fig. 19E2

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

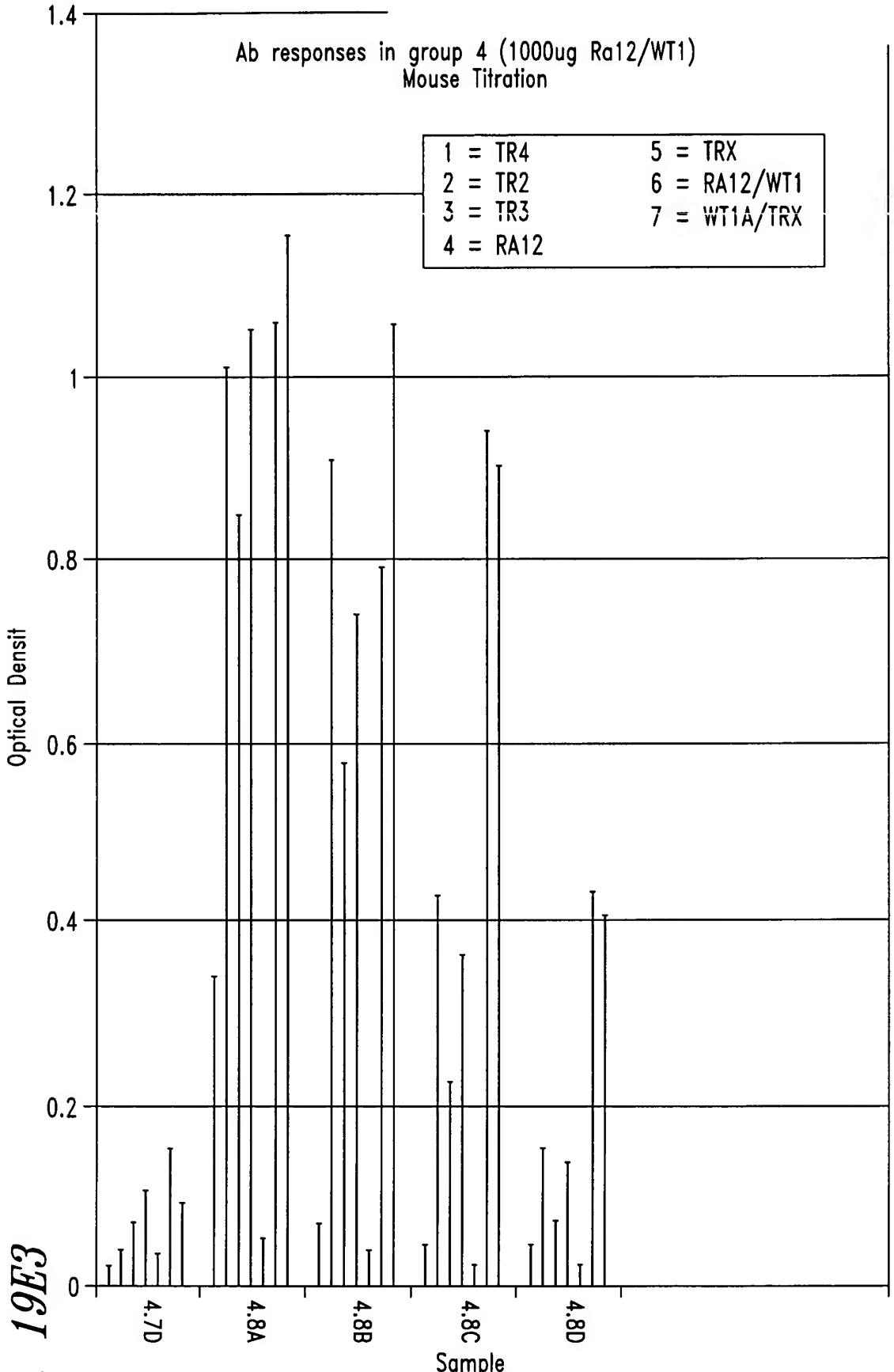


Fig. 19E3

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

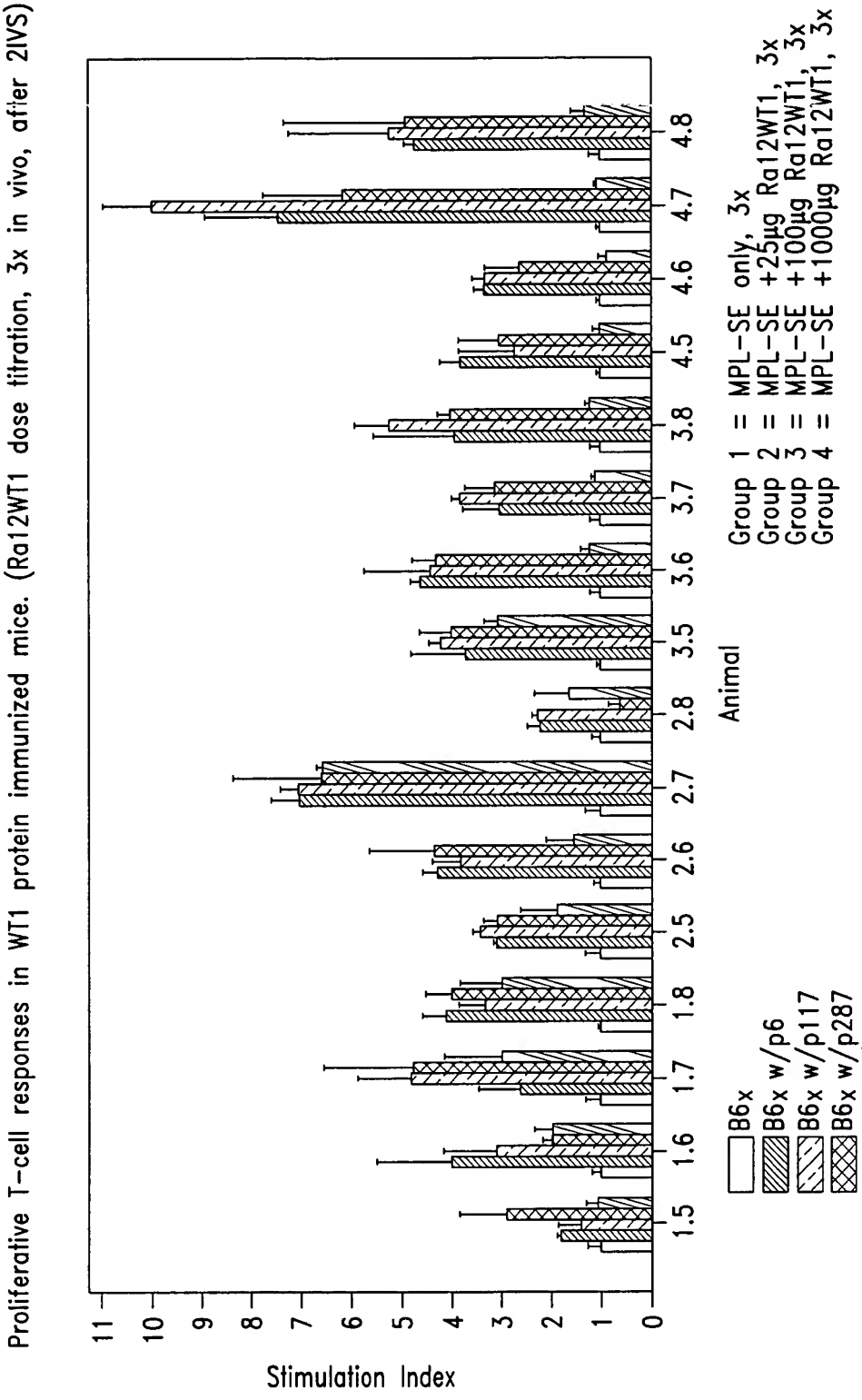

Proliferative T-cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 3x in vivo, after 2IVS)
Stimulation Index
0
1
2
3
4
5
6
7
8
9
10
11
Animal
1.5
1.6
1.7
1.8
2.5
2.6
2.7
2.8
3.5
3.6
3.7
3.8
4.5
4.6
4.7
4.8
B6x
B6x w/p6
B6x w/p117
B6x w/p287
B6x w/p421
Group 1 = MPL-SE only, 3x
Group 2 = MPL-SE +25µg Ra12WT1, 3x
Group 3 = MPL-SE +100µg Ra12WT1, 3x
Group 4 = MPL-SE +1000µg Ra12WT1, 3x

Fig. 20A

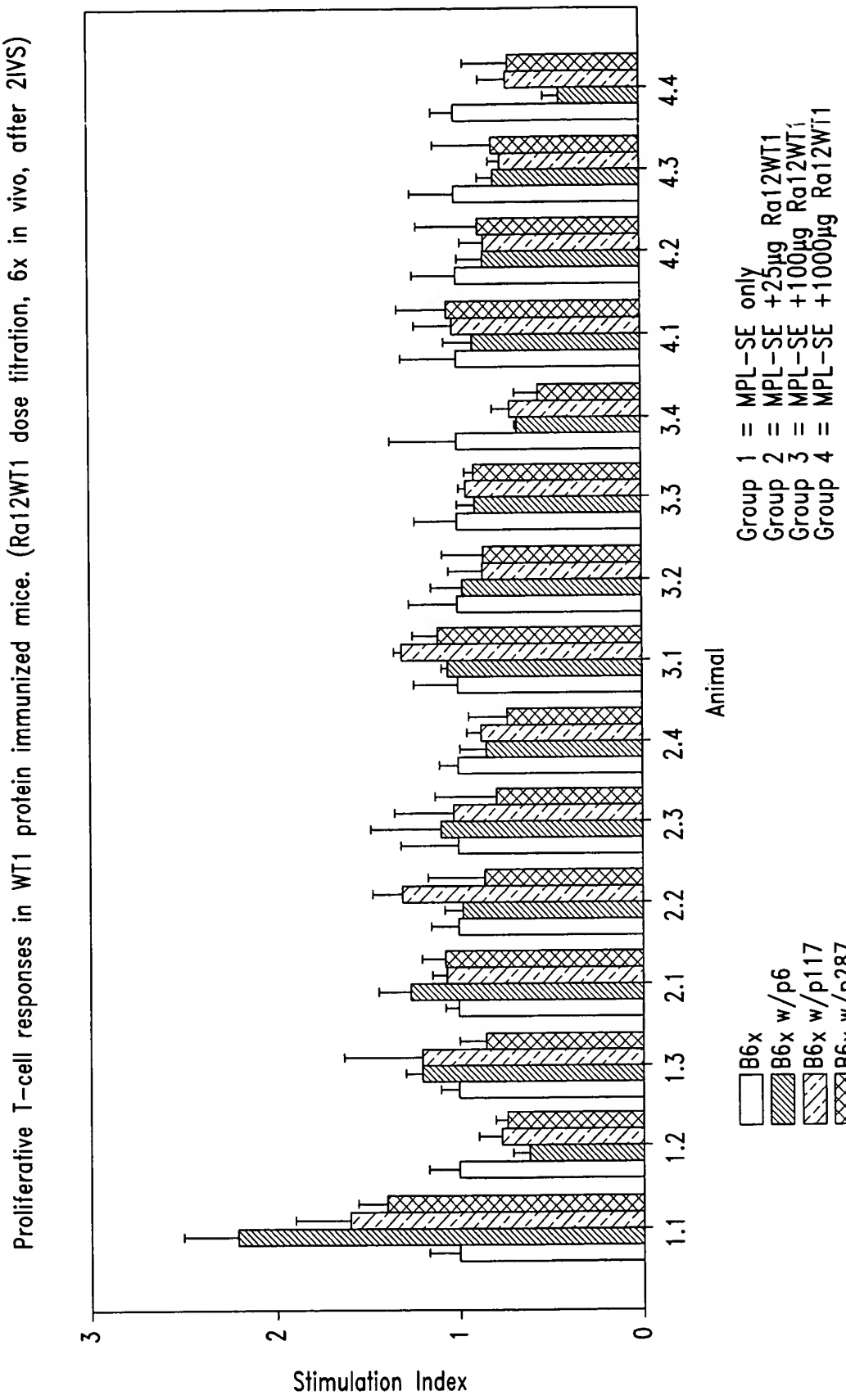

Proliferative T-cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 6x in vivo, after 2IVS)
Stimulation Index
0
1
2
3
Animal
1.1
1.2
1.3
2.1
2.2
2.3
2.4
3.1
3.2
3.3
3.4
4.1
4.2
4.3
4.4
B6x
B6x w/p6
B6x w/p117
B6x w/p287
Group 1 = MPL-SE only
Group 2 = MPL-SE +25µg Ra12WT1
Group 3 = MPL-SE +100µg Ra12WT1
Group 4 = MPL-SE +1000µg Ra12WT1

Fig. 20B

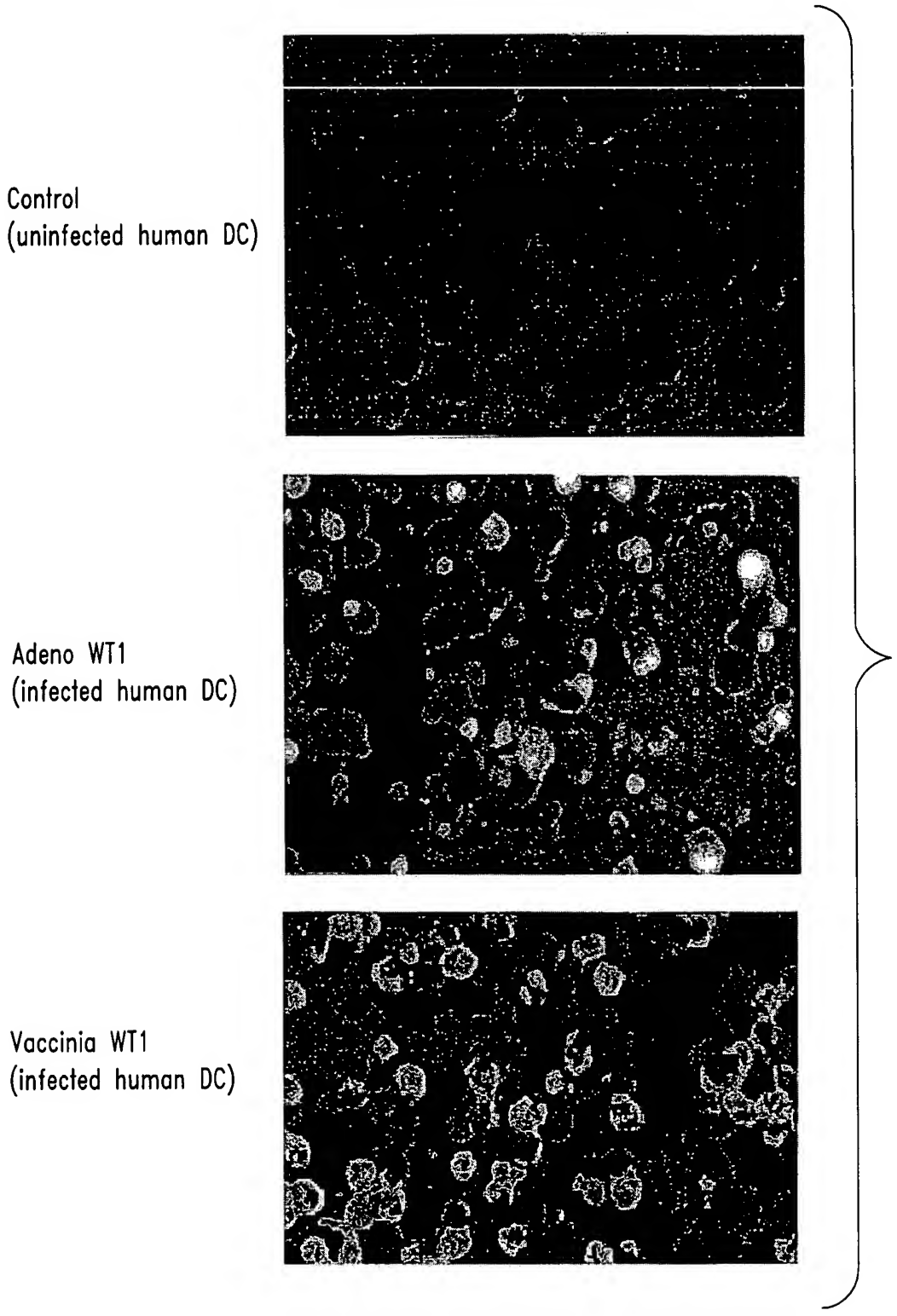
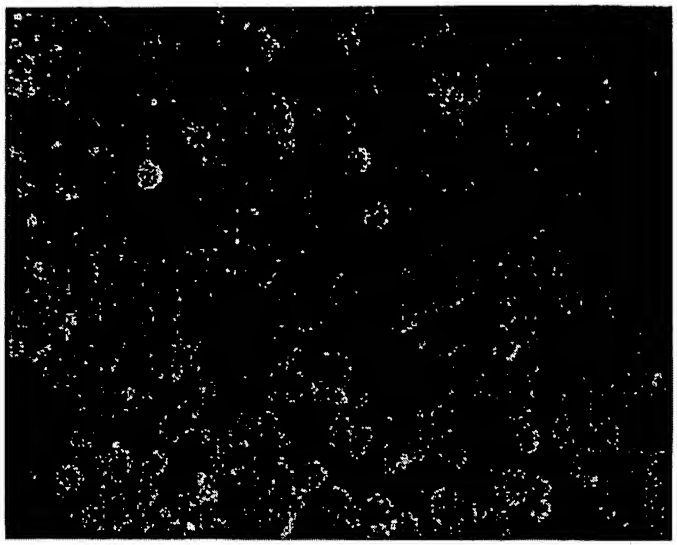

WT1 expression in human DC following adeno WT1 and Vaccinia WT1 infection
Control (uninfected human DC)
Adeno WT1 (infected human DC)
Vaccinia WT1 (infected human DC)

Fig. 21

WT1 can be expressed reproducible in human DC following adeno WT1 infection and is not induced by a control Adeno infection

Control (Adeno EGFP infected human DC)



Vaccinia WT1 (infected human DC)

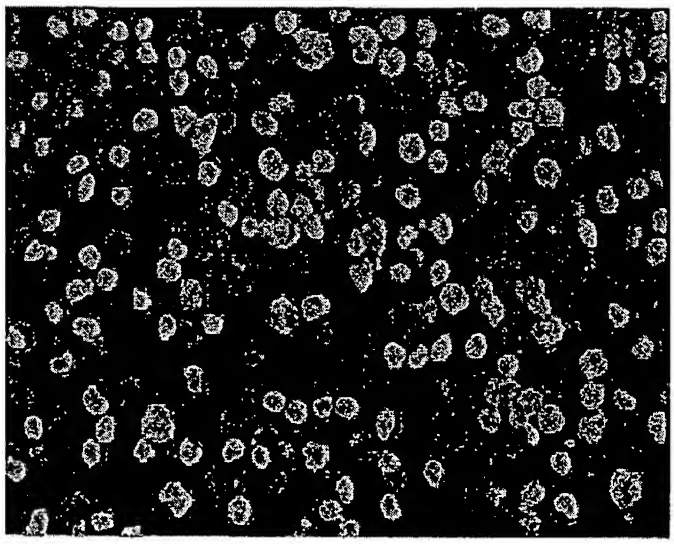


Fig. 22

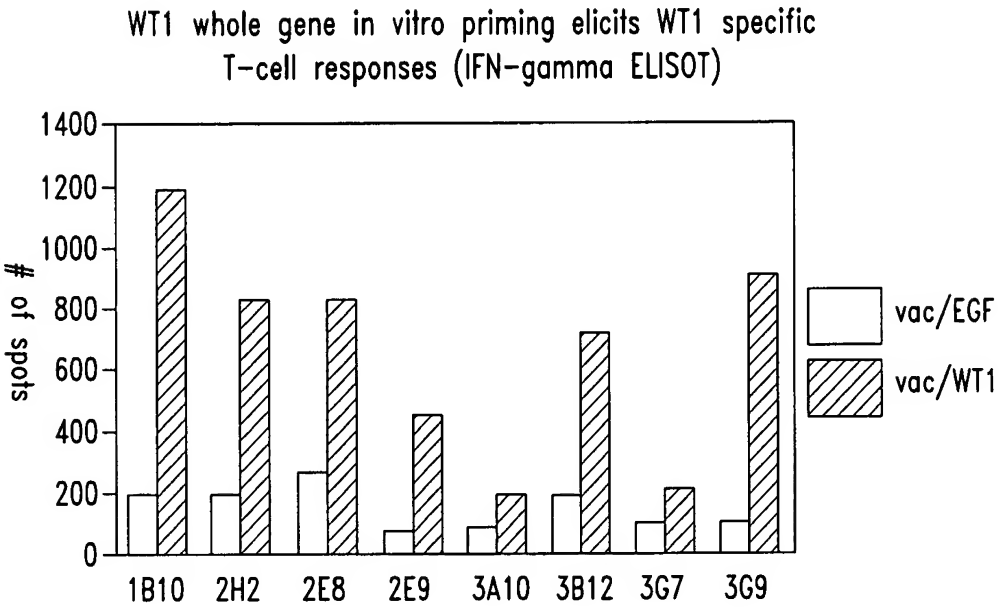

WT1 whole gene in vitro priming elicits WT1 specific
T-cell responses (IFN-gamma ELISOT)
# of spots
1400
1200
1000
800
600
400
200
0
1B10
2H2
2E8
2E9
3A10
3B12
3G7
3G9
vac/EGF
vac/WT1

Fig. 23

WT-1 F cDNA 2-281

GGCTCCGACGTTCGGGACCTGAACGCACTGCTGCCGGCAGTTCCGTCCCTGGGTGGTGGTGGTGGTTGCGCACTGCCGGTTAGCGGTGCAGCACAGTGGG
CTCCGGTTCTGGACTTCGCACCGCCGGGTGCATCCGCATACGGTTCCCTGGGTGGTCCGGCACCGCCGCCGGCACCGCCGCCGCGCCGCCGCGCCGCC
GCACTCCTTCATCAAACAGGAACCGAGCTGGGGTGGTGCAGAACCGCACGAAGAACAGTGCCTGAGCGCATTCACCGTTCACTTCTCCGGCCAGTTCACT
GGCACAGCCGGAGCCTGTCGCTACGGGCCCTTCGGTCCTCCTCCGCCCAGCCAGGCGTCATCCGGCCAGGCCAGGATGTTTCCTAACGCGCCCTACCTGC
CCAGCTGCCTCGAGAGCCAGCCCGCTATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACGCCCAGCTACGGTCACACGCCCTCGCACCATGC
GGCGCAGTTCCCCAACCACTCATTCAAGCATGAGGATCCCATGGGCCAGCAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTCTATGGC
TGCCACACCCCCACCGACAGCTGCACCGGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGACAATTTATACCAAATGACATCCCAGCTTGAAT
GCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGCCACAGCACAGGGTACGAGAGCGATAACCACACAACGCCCATCCTCTGCGGAGCCCA
ATACAGAATACACACGCACGGTGTCTTCAGAGGCATTCAGTGA

WT-1 F amino acid 2-281

GSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFT
GTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYG
CHTPTDSCTGSQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQ

Fig. 24